Docket No. 1010/101US14

IN THE UNITED STATES PATENT AND TRADEMARK OFFICE

In re Application of:	Leo J. Romanczyk, Jr. and Harold H. Schmitz		
Filed:	February 17, 2004	Group Art Unit:	1626
Serial No:	10/780,298	Examiner:	T. Solola
For:	Compositions for, and Methods of, Anti-Platelet Therapy		

DECLARATION BY REBECCA J. ROBBINS

Commissioner for Patents
P. O. Box 1450
Alexandria, VA 22313-1450

1. My name is Rebecca J. Robbins. I am a Research Chemist at Masterfoods USA, 800 High Street, Hackettstown, NJ 07840.
2. I am an employee of Mars, Incorporated [hereinafter "Mars"] and receive no special benefits from the execution of this declaration.
3. I have obtained a Ph.D. degree in Organic Chemistry from the University of Maryland, College Park, MD, U.S.A. A copy of my Curriculum Vitae is enclosed (Attachment 1).
4. My research interests are in the field of polyphenol chemistry and include research in the field of analysis of polyphenols including development of analytical methods for measuring polyphenols, and methods of isolation and characterization of polyphenols. I have also taught organic chemistry (University-level) for several years. I am knowledgeable about the experiments with polyphenol compounds conducted by Mars and the obtained results. I am also knowledgeable about the published literature and suitable experimental techniques in this area of research.

5. I am not an inventor of the above-identified patent application nor do I derive any interest from it.
6. I have reviewed the Official Action mailed by the US Patent Office on January 23, 2006 in the above-identified patent application (pages 2-3), pending claims (particularly rejected claims 35 and 80), and the text of the application (particularly page 8, 3rd paragraph, which the Examiner mentions in the Official Action).
7. I was asked by the attorney for Mars whether a chemist knowledgeable in the field of synthetic organic chemistry would have been able to make gallated and glycosylated derivatives of procyanidin oligomers recited in claims 35 and 80 as of April 2, 1996, which I understand to be the effective filing date of this application.
8. As of April 2, 1996, the terminology "gallate esters", "gallated compound", "glycosylation", and "glycosylated compound" was well accepted and widely used in the field of synthetic organic chemistry and related fields (as evidenced, for example, by the attached publications).
9. Thus, a chemist working in the area of synthetic organic chemistry would have recognized that the term "gallated procyanidin oligomer" recited in claim 35 refers to a procyanidin oligomer having one or more gallic acid (*i.e.*, 3,4,5-trihydroxybenzoic acid; also referred to as a "galloyl" group) substituents added to the procyanidin.
10. A synthetic organic chemist would have also recognized that the term "glycosylated procyanidin oligomer" recited in claim 80 (which can also be referred to as glycoside) refers to a procyanidin oligomer having one or more sugar (carbohydrate) substituent(s). The reaction of glycosylation encompasses "glucuronidation" which is the addition of a glucuronic acid (carboxylic acid of glucose) molecule to another compound, e.g. a procyanidin.
11. Methods of making gallated and glycosylated compounds were well known as of April 2, 1996.
12. Natural as well synthetic routes of obtaining gallated compounds were readily available and standard as of April 2, 1996. Gallate esters of polyphenols are

encountered commonly in nature and could have been prepared by extraction from such sources, for example, such esters of procyanidins are commonly found in the rhubarb plant (a rhizome). For example, see, Yokozawa *et al.*, *Nephron*, 1991, 58(2):155-60 (Attachment 2), which article shows that gallates of procyanidin B-2 and procyanidin C-1 are present in and can be isolated from rhubarb (*see* figure 1 on page 156 for structural formulae of gallate esters of procyanidins B-2 and C-1 and "Materials and Methods" for isolation procedure).

13. Chemical synthesis of gallated procyanidin oligomers could have been achieved *via* a standard esterification reaction using gallic acid and procyanidin oligomer as starting material. Gallate esters of polyphenols (including procyanidins) could have been formed *via* a classical esterification reaction, for example, involving the –COOH group of gallic acid and any of the –OH groups of the polyphenol ring(s). For example, an esterification reaction between the –OH group on position 3 of any monomeric unit of a procyanidin and the –COOH group of gallic acid will result in a gallated procyanidin oligomer. The Fischer esterification reaction, which has been well-known for over 70 years, exemplifies such a reaction. For example, *see*, McMurry J., "*Organic chemistry*" (textbook), 1992 (Attachment 3, page 804, figure 21.6) which chapter shows the mechanism of Fischer esterification. *See also,* page 805 in Attachment 3 which shows the synthesis of methyl benzoate (an ester) from benzoic acid and methanol via Fischer esterification. Thus, gallated procyanidin oligomers could have been easily envisioned and prepared by a synthetic organic chemist in the field of polyphenol synthesis as of April 2, 1996.
14. Attaching sugar residues (glycosylation) to a procyanidin oligomer could have been achieved using standard chemical techniques. Several methods of making glycosylated compounds were known as of April 2, 1996 and are discussed in paragraph 15.
15. The attachment of sugar residue(s) to phenol-type compounds and various methods for producing glycosides in stereoselective manner were well known in April 1996. For example, *see* Scheme I in Bellamy *et al.*, *J. Med. Chem.*, 1993, 36(7):898-903 (Attachment 4, page 899, and references cited therein, e.g. ref. no.

11). Bellamy shows three methods of making glycosylated derivatives of benzophenone (a polyphenol-like compound), including the classical Koenings-Knorr reaction for the glycosylation of phenols (Attachment 4, page 898, par. spanning two columns). *See also* Scheme 29 in Danishefsky and Bilodeau, *Angew. Chem. Ed. Engl.*, 1996, 35:1380-1419 (Attachment 5, page 1394), which article shows another method of making a glycosylated derivative of a phenol-like compound. Thus, glycosylated procyanidin oligomers could have been easily envisioned and prepared by a synthetic organic chemist in the field of polyphenol synthesis as of April 2, 1996.

16. In conclusion, as of April 2, 1996, a synthetic organic chemist would have been able to envision gallated and glycosylated procyanidin oligomers and prepare such compounds using the methods known at the time such as those referenced above and in the attached publications.

17. I declare that the above statements are true to the best of my knowledge.

Date: 4/14/06

Rebecca J. Robbins, Ph.D.

REBECCA J. ROBBINS, Ph.D.

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EDUCATION:

Ph.D. Degree in Organic Chemistry University of Maryland, College Park, MD, 1995
B.A. Degree in Philosophy Summa cum Laude, Phi Beta Kappa, Brooklyn College, Brooklyn, NY 1989

WORK EXPERIENCE:

Research Chemist in the Analytical and Applied Services Division **2004-present**
MasterfoodsUSA, Division of Mars, Inc. Hackettstown, NJ

- Analytical method development for the determination of flavanols and procyanidins in foods as well as for larger scale physical isolation of polyphenols (purification of polyphenols from food sources). Characterization of flavanols and procyanidins using techniques such as LC/MS.

Research Chemist **2001-2004**
Food Composition Laboratory, USDA, Beltsville Agricultural Research Center Beltsville, MD

- As lead scientist, established a research program for the determination of phenolic phytonutrients in foods with emphasis on developing analytical methodology (quantitative and qualitative). Research included quantitative investigations on sample preparation and extraction procedures.
- Assembled research team, led recruitment efforts resulting in the hiring of one support scientist and two research associates, and developed a solid, focused research plan coordinating the expertise of participants on the project.
- Obtained major funding (for a 5-year plan) through a competitive Agricultural Research Service-USDA program (independent and external expert review process).

Visiting Assistant Professor-Lecturer **2000-2001**
Chemistry Department, Vassar College, Poughkeepsie, NY

- Taught organic chemistry courses (lecture and laboratory). Final grade responsibility.
- Designed molecular modeling laboratory experiment and established resource web page for students (organic chemistry learning tools).

Laboratory Instructor - Chemistry Department **1998-1999**
Tulane University, New Orleans, LA

- Independently directed organic laboratory courses. Supervised and directed five teaching assistants. Instructed students in laboratory experiments, prepared laboratory assignments and administered exams.
- Instructed students in laboratory experiments, prepared laboratory assignments and administered exams.
- Initiated collaboration with Tulane Computing Services to implement an Internet lab experiment, with Environmental Health and Safety Office to organize a safety seminar for students, and Tulane chemistry department professors for the design of a 'green' organic synthesis experiment (water-based nucleophilic addition).

General and Organic Chemistry Tutor: Self-Employed. **1998-1999**
New Orleans, LA

- Instituted and initiated a tutoring service: Individual and group instruction of 20-25 students a week. Wrote study guides and sample exams to assist in clarifying concepts.

Post-Doctoral Research Associate **1996-1998**
Chemistry Department, Tulane University, New Orleans, LA

- Worked on project "Oxidation of Olefins via Singlet Oxygen within Organic Dye exchanged Zeolites", with Dr. V. Ramamurthy. Determined selectivity of the oxidation of olefins (via singlet oxygen) within the confines of zeolite supercages. Techniques employed were GC, GC/MS, NMR. Researched and co-authored five publications on Zeolite topics.

Graduate Studies: Teaching and Research Assistant **1990-1995**
Department of Chemistry and Biochemistry, University of Maryland, College Park, MD

- Thesis title: "Photogenerated Reactive Intermediates: Radical Anions and Arylnitrenium Ions" with Dr. Daniel E. Falvey. Investigated the substituent effects on lifetimes and selectivities of photogenerated arylnitrenium ions via transient laser spectroscopy and product isolation. Co-authored six publications.
- Synthesized a series of anthranilium salts photoprecursors, purified compounds via column and radial chromatographies and recrystallization, confirmed structures via NMR (^{13}C, ^{1}H), MS, IR and UV.
- Received an award as recognition for excellence in academic presentation.

PUBLICATIONS

"High-performance liquid chromatography separation and purification of cocoa (Theobroma cacao L.) procyanidins according to the degree of polymerization using a diol stationary phase." Kelm, M.A., C.J. Johnson, R.J. Robbins, J.F. Hammerstone, and H.H. Schmitz in *Journal of Agricultural and Food Chemistry* **2006** 54:1571-1576.

"Optimization of extraction process for phenolic compounds from Black Cohosh " Sudarsan Mukhopadhyay, Devandand L. Luthria and Rebecca J. Robbins *J Sci of Food and Agriculture*, **2006**, 86 156-162.

"Selenium enrichment of broccoli: interactions between selenium and secondary plant compounds" John W. Finley, Anna Sigrid-Keck, Rebecca J. Robbins and Korry J. Hintze *J. of Nutrition* **2005**, 135: 1236-1238.

"Cultivation conditions and selenium fertilization alter the phenolic profile and sulforaphane content of broccoli" Rebecca J. Robbins, Anna-Sigrid Keck, Gary Banuelos, John. W. Finley, *Journal of Medicinal Foods*, **2005**, Vol. 8, No. 2: 204-214.

"Optimized synthesis of four isotopically labeled (^{13}C-enriched) phenolic acids via a malonic acid condensation" Rebecca J. Robbins and Walter F. Schmidt, *Journal of labeled compounds and radiopharmaceuticals*. **2004**, 47, 797-806.

"Development of a measurement system for phenolic acids: Quantitative High-performance Liquid Chromatography with Photodiode Array Detection". Rebecca J. Robbins and Scott R. Bean, *Journal of Chromatography A*, **2004**,1038, 97-105.

"Phenolic Acids in Food- An Overview in Analytical Methodology" Rebecca J. Robbins Review Article in *Journal of Agricultural and Food Chemistry*, **2003**, 51, 2866-2887.

"Cation Controlled Singlet Oxygen Mediated Oxidation of Olefins with Zeolites" Lakshmi S. Kaanumalle, J. Shailaja, Rebecca J. Robbins, V. Ramamurthy. *J. Photochem & Photobio.A* **2002**, 153, 55-65.

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"Detection and Estimation of Brønsted Acid Sites Within Na+Y and Na+X Zeolites" V. Jayathirtha Rao, Deborah Perlstein, Rebecca J. Robbins, P. H. Laksminarashimhan, Hsein-ming Kao, Clare P. Grey and V. Ramamurthy *Chem Comm.* **1998**, 269.

"Asymmetrically Modified Zeolite as a Medium for Enantioselective Photoreactions: Reactions from Spin Forbidden Excited States" Abraham Joy, Rebecca J. Robbins, Kasi Pitchumani and V. Ramamurthy *Tetrahedron Lett.* **1997**, 51, 8825.

"Generation and Reactivity of Singlet Oxygen Within Zeolites: A Remarkable Control on the Schenk (Ene) Reaction" Rebecca J. Robbins and V. Ramamurthy *Chem Comm.* **1997**, 107.

"Substituent Effects on the Lifetimes and Reactivities of Arylnitrenium Ions Studied by Laser Flash Photolysis and Photothermal Beam Deflection " Rebecca J. Robbins, David Laman and Daniel E. Falvey. *J. Am. Chem. Soc.* **1996**, 118, 8127-8135.

"Photochemically Generated Arylnitrenium Ions: Laser Flash Photolysis and Product Studies of the Photochemistry of N-tert-Butyl-3-methylanthranilium Ions." Dominic Chiapperino, Gary B. Anderson, Rebecca J. Robbins, and Daniel E. Falvey. *J. Org. Chem.* **1996**, 61, 3195-3199.

"Substituted Arylnitrenium Ions: Reactivity and Selectivity of N-tert-Butyl (2-Acetyl-4-substituted) Phenyl Nitrenium Ions Towards Alcohols and Water" Rebecca J. Robbins, Lucie L-N. Yang, Gary B. Anderson, and Daniel E. Falvey. *J. Am. Chem. Soc.* **1995**, 117, 6344.

"Photogenerated Nitrenium Ions: Singlet and Triplet State Reactions of tert-Butyl-(2-acetyl4-methyl)phenyl Nitrenium Ion" Rebecca J. Robbins and Daniel E. Falvey *Tetrahedron Lett.* **1994**, 35, 4943.

"Stereochemistry of the Solid-State Photodimerization of Thymoquinone" Rebecca J. Robbins and Daniel E. Falvey *Tetrahedron Lett.*, **1993**, 34, 3509.

"Radical Anion Reactions of Cyclobutane Derivatives: Electron Transfer Cleavage of Dithymoquinone" Rebecca J. Robbins and Daniel E. Falvey *J. Org. Chem.* **1993**, 58, 3616.

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Effects of Rhubarb Tannins on Uremic Toxins

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Key Words. Tannin · (−)-Epicatechin 3-O-gallate · Procyanidin B-2 3,3′-di-O-gallate · Rhubarb · Uremic toxin · Renal failure

Abstract. The effects of each of several tannins purified from Rhei Rhizoma on serum constituents were investigated in rats with adenine-induced renal failure. Blood levels of urea nitrogen, methylguanidine (MG), and guanidinosuccinic acid (GSA) were significantly decreased in rats given (−)-epicatechin 3-O-gallate at a dose of 2.5, 5, or 10 mg/kg body weight/day for 24 days. The creatinine (Cr) level was also significantly decreased in rats given 5 and 10 mg of this compound. A significant decrease in urea nitrogen, MG, and GSA was found in rats given 6.25 mg procyanidin B-2 3,3′-di-O-gallate. However, unlike the former two components, the administration of 12.5 mg of procyanidin C-1 3,3′,3″-tri-O-gallate produced a considerable or significant increase in blood levels of urea nitrogen, Cr, MG, and GSA. RG-tannin had a weaker overall effect on serum constituents except for GSA in comparison with the corresponding effect of (−)-epicatechin 3-O-gallate and 6.25 mg of procyanidin B-2 3,3′-di-O-gallate. Rhatannin tended to increase the serum nitrogen constituents.

Introduction

In recent years, pharmacological and biochemical studies of rhubarb, which is used extensively in Chinese medicine, have been carried out in parallel with chemical studies, in order to clarify the active constituent of this plant [1, 2]. In a previous experiment, we administered an aqueous extract of rhubarb orally to rats for several consecutive days together with a 0.75% adenine diet, or after induction of renal failure. Blood levels of urea nitrogen, creatinine (Cr), methylguanidine (MG), and guanidinosuccinic acid (GSA) decreased in a dose-dependent manner, suggesting that the rhubarb aqueous extract ameliorated the severity of renal failure [3, 4]. The examination of the active constituent of this drug revealed that the activity was contained in fraction II-3, obtained by purification of the aqueous extract through Sephadex LH-20 and MCI-gel CHP 20P columns. The activity was also found to be transferred from fraction II-3 to the aqueous layer of fraction II-3-2, which was obtained by further purification of fraction II-3. It was speculated that this fraction contained tannins composed of procyanidin oligomers linked with gallates [5]. In the present study, the effects of five different tannins on blood components were investigated. These five tannins are obtained from rhubarb at relatively high percentage yields and differ from one another in the degree of galloylation of the constituent unit, (−)-epicatechin 3-O-gallate.

Materials and Methods

Animals and Treatments

Male rats of the LWH: Wistar strain, with a body weight of 200–210 g, were kept in an animal room at an ambient temperature of 23 ± 1 °C with lights on from 06.00 to 18.00 h. They were allowed an adaptation period of several days, during which they were fed on a commercial feed (type CE-2, CLEA Japan Inc., Tokyo, Japan). They were then fed ad libitum on an 18% casein diet containing 0.75% adenine, which produced experimental renal failure in the animals. In rats with renal failure induced by adenine, renal impairment becomes aggravated as the period of adenine feeding increases. It was previously confirmed by histological and biochemical proce-

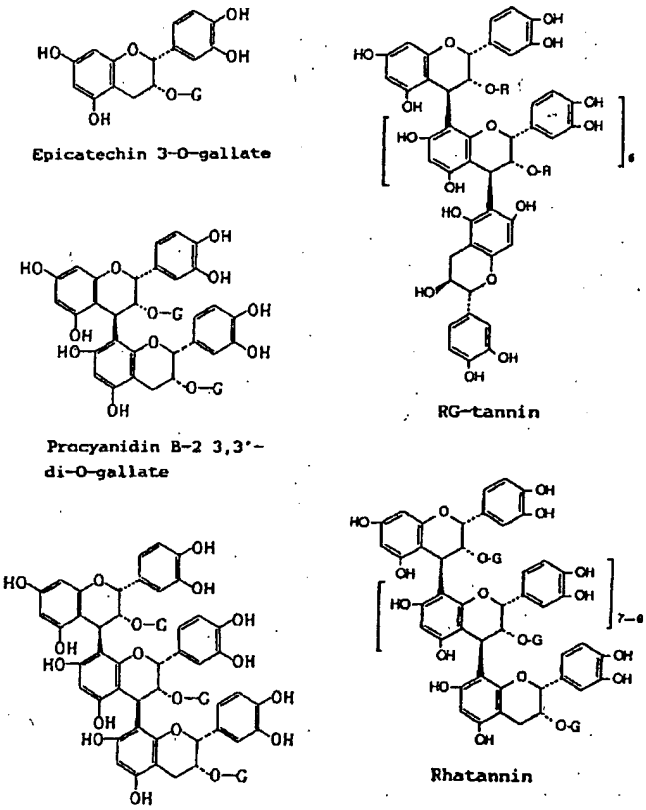

Fig. 1. Structural formulae of tannins.

dures that renal failure was present after 6 days of ingestion [6–12]. During the adenine-feeding period, an aqueous solution of tannin was administered orally for 24 days as drinking water, while control rats received tap water.

In the present study, doses of 2.5, 5, and 10 mg/kg body weight/day were used for (−)-epicatechin 3-O-gallate, 6.25 and 12.5 mg/kg body weight/day for procyanidin B-2 3,3'-di-O-gallate, and 3.125 and 12.5 mg/kg body weight/day for procyanidin C-1 3,3',3"-tri-O-gallate. RG-tannin and rhatannin were administered orally at doses of 7.5 and 15 mg/kg body weight/day, respectively. Throughout the experimental period, there were no statistically significant differences between the controls and the rats treated with each tannin with regard to changes in body weight. The food intake of each rat was essentially proportional to weight change. No case of diarrheal symptoms was found. Six rats were used for each experimental group. Values are expressed as means ± SE.

Isolation of Tannin

Roots of *Rheum officinale* Baillon obtained in China (3.2 kg) were finely powdered and extracted five times with 80% aqueous acetone at room temperature. The extract, after removal of acetone by evaporation under reduced pressure, was subjected to chromatography over Sephadex LH-20. Elution with H_2O containing increasing proportions of MeOH yielded six fractions (I–VI), which consisted of relatively lower-molecular-weight phenolics. Further elution with H_2O-acetone (1:1) gave a fraction (VII) containing procyanidin polymers. Fraction IV (140 g) was rechromatographed over Sephadex LH-20 with EtOH and MCI-gel CHP 20P with H_2O-MeOH (1:0→1:1) to yield (−)-epicatechin 3-O-gallate (11.0 g) [13]. Repeated chromatography of fractions V (160 g) and VI (400 g) over Sephadex LH-20 (EtOH), MCI-gel CHP 20P [H_2O-MeOH (1:0→1:1)] and Fuji gel ODS G3 [H_2O-MeOH (1:0→1:1)] afforded procyanidins B-2 3,3'-di-O-gallate (9.8 g) [13] and C-1 3,3',3"-tri-O-gallate (2.9 g) [14], respectively. Fraction VII (150 g) was separated by MCI-gel CHP 20P [H_2O-MeOH (1:0→1:1)] with monitoring by HPLC to give RG-tannin (~40 g) and rhatannin (~60 g) [13]. The chemical structures of the compounds used in this experiment are shown in figure 1.

Analyses

On day 24 of the administration period, rats were stunned by a sharp blow on the head. Blood was collected into a conical centrifuge tube, and the serum was separated immediately by centrifugation for determinations of urea nitrogen, Cr, MG, and GSA. Urea nitrogen was determined using a commercial reagent (BUN Kainos, obtained from Kainos Laboratories, Inc., Tokyo, Japan). For determinations of Cr, MG, and GSA, the serum was deproteinized by addition of trichloroacetic acid (final concentration, 10%). The supernatant obtained by centrifugation at 3,000 rpm for 10 min was injected into a Japan Spectroscopic liquid chromatograph using a step-gradient system. A fluorescence spectrometer (excitation 365 nm, emission 495 nm; model FP-210, Japan Spectroscopic Co., Tokyo, Japan) was used for the detection of the substances on the column.

Statistics

The significances of differences between the control and tannin-treated groups were tested using Student's t test.

Results

(−)-Epicatechin 3-O-gallate

Table 1 shows the effect of (−)-epicatechin 3-O-gallate on serum constituents. The administration of (−)-epicatechin 3-O-gallate to rats resulted in a decrease in urea nitrogen; there was a 24% decrease in the 2.5-mg group, and a 27% decrease in the 5-mg group, both being statistically significant. Similar changes produced by (−)-epicatechin 3-O-gallate administration were observed in the 10-mg group. The Cr value tended to be decreased in rats given 2.5 mg. The Cr value in rats given 5 and 10 mg was significantly decreased, by 16 and 20% of the level in control rats, respectively. In contrast, the MG value was significantly lower, by 37, 34, and 27%, in rats given 2.5, 5, and 10 mg of (−)-epicatechin 3-O-gallate, respectively. The GSA values were also markedly and significantly decreased in rats given this constituent, the value being 47% lower in the 2.5-mg group and 61% lower in the 10-mg group in comparison with the control group.

Table 1. Effect of (-)-epicatechin 3-O-gallate on serum constituents

Group	Dose mg/kg BW/day	Urea nitrogen mg/dl	Cr mg/dl	MG μg/dl	GSA μg/dl
Control	-	125.1 ± 12.4	3.64 ± 0.15	11.19 ± 1.39	114.21 ± 12.98
(-)-Epicatechin 3-O-gallate	2.5	95.7 ± 6.7*	3.25 ± 0.18	7.03 ± 0.85*	60.79 ± 11.34**
(-)-Epicatechin 3-O-gallate	5	90.8 ± 5.3*	3.06 ± 0.10**	7.40 ± 0.68*	49.38 ± 6.82**
(-)-Epicatechin 3-O-gallate	10	91.2 ± 2.9*	2.92 ± 0.19**	8.12 ± 0.89*	44.54 ± 4.90***

*$p < 0.05$; **$p < 0.01$; ***$p < 0.001$, i.e. significantly different from control value.

Table 2. Effect of procyanidin B-2 3,3'-di-O-gallate on serum constituents

Group	Dose mg/kg BW/day	Urea nitrogen mg/dl	Cr mg/dl	MG μg/dl	GSA μg/dl
Control	-	117.0 ± 8.7	3.63 ± 0.22	14.96 ± 2.07	135.74 ± 17.55
Procyanidin B-2 3,3'-di-O-gallate	6.25	97.1 ± 4.3*	3.24 ± 0.23	8.13 ± 1.09**	88.87 ± 8.10**
Procyanidin B-2 3,3'-di-O-gallate	12.5	128.0 ± 7.9	3.85 ± 0.10	8.59 ± 1.16**	155.98 ± 3.94

*$p < 0.05$; **$p < 0.01$, i.e. significantly different from control value.

Table 3. Effect of procyanidin C-1 3,3', 3"-tri-O-gallate on serum constituents

Group	Dose mg/kg BW/day	Urea nitrogen mg/dl	Cr mg/dl	MG μg/dl	GSA μg/dl
Control	-	105.5 ± 7.5	3.34 ± 0.23	10.64 ± 1.32	106.49 ± 7.80
Procyanidin C-1 3,3', 3"-tri-O-gallate	3.125	104.4 ± 5.0	3.61 ± 0.16	10.59 ± 1.60	114.28 ± 7.81
Procyanidin C-1 3,3', 3"-tri-O-gallate	12.5	144.6 ± 6.6*	5.43 ± 0.53*	14.23 ± 1.34	137.53 ± 15.68

*$p < 0.01$, i.e. significantly different from control value.

Procyanidin B-2 3,3'-di-O-gallate

Table 2 shows the effect of procyanidin B-2 3,3'-di-O-gallate on parameters of serum constituents after administration of oral doses of 6.25 and 12.5 mg/kg body weight/day. The urea nitrogen value showed a significant decrease in rats given 6.25 mg of procyanidin B-2 3,3'-di-O-gallate, whereas the further increase in the dose to 12.5 mg produced no differences in urea nitrogen levels between the control and procyanidin B-2 3,3'-di-O-gallate-treated groups. The Cr value tended to be decreased in rats given 6.25 mg. The MG values were 46 and 43% lower in rats given 6.25 mg and 12.5 mg, respectively, than in control rats, showing a greater effect of procyanidin B-2 3,3'-di-O-gallate. Although the GSA value decreased significantly, by 35%, in rats given 6.25 mg; the administration of 12.5 mg showed a slight increase.

Procyanidin C-1 3,3',3"-tri-O-gallate

Although the dose of 3.125 mg produced no differences in urea nitrogen between the control and procyanidin C-1 3,3',3"-tri-O-gallate-treated groups, the further increase in the dose to 12.5 mg significantly increased the urea nitrogen value, from 105.5 to 144.6 mg/dl (a 37% change, $p < 0.01$), as shown in table 3. Similar changes produced by procyanidin C-1 3,3',3"-tri-O-gallate administration were observed in Cr. Both the MG and GSA

Table 4. Effect of RG-tannin on serum constituents

Group	Dose mg/kg BW/day	Urea nitrogen mg/dl	Cr mg/dl	MG μg/dl	GSA μg/dl
Control	–	103.2±8.4	3.24±0.13	11.47±1.57	96.06±13.78
RG-tannin	7.5	98.8±9.4	3.05±0.15	10.44±1.24	50.79±5.75*
RG-tannin	15	94.3±7.8	3.10±0.10	10.30±0.62	42.79±7.03**

*$p<0.05$; **$p<0.01$, i.e. significantly different from control value.

Table 5. Effect of rhatannin on serum constituents

Group	Dose mg/kg BW/day	Urea nitrogen mg/dl	Cr mg/dl	MG μg/dl	GSA μg/dl
Control	–	107.2±5.4	3.34±0.17	11.93±1.14	92.81±11.39
Rhatannin	7.5	102.6±6.6	3.39±0.06	12.92±0.70	114.35±11.54
Rhatannin	15	128.0±6.1*	3.52±0.09	12.79±1.70	121.71±20.35

*$p<0.05$, i.e. significantly different from control value.

values in rats given 12.5 mg also showed a considerable increase, although neither increase was statistically significant.

RG-tannin

Table 4 shows the effect of RG-tannin on parameters of serum constituents after administration of oral doses of 7.5 and 15 mg/kg body weight/day. Although there were no particular variations in the urea nitrogen, Cr, and MG values after RG-tannin administration, the GSA value was markedly and significantly decreased in rats given this compound, the value being 47% lower in the 7.5-mg group and 55% lower in the 15-mg group in comparison with the control group.

Rhatannin

As shown in table 5, the urea nitrogen level was 107.2 mg/dl in the controls, whereas the value was significantly higher, by 19%, in rats given 15 mg of rhatannin. The GSA values also showed a considerable (but not significant) increase in rats given 7.5 and 15 mg of rhatannin. However, there were no significant differences in the Cr and MG values between the control and rhatannin-treated groups, at either the 7.5- or 15-mg dosage level.

Discussion

In recent years, much attention has been focused on various medicinal actions of rhubarb prescribed in Chinese medicine, apart from its laxative action. These actions were analyzed pharmacologically and biochemically as well as from a chemical aspect, and many new constituents were isolated and their structures identified. It is now clear that rhubarb has a laxative action due to its sennoside constituent, antibiotic and antitumor actions due to anthraquinones, antihyperlipemic and liver-function-improving actions due to stilbenes, and analgesic and anti-inflammatory actions due to lindlein [1, 2, 15].

On the other hand, we have carried out extensive studies using rats with experimental renal failure in order to analyze prescriptions of Oriental medicine, in order to investigate the physiologically and pharmacologically active substances contained in crude drugs, and to elucidate their action mechanisms, aiming at their clinical application. Through these studies, we have found that rhubarb causes a decrease in urea nitrogen as well as a marked decrease in blood levels of nitrogen-containing compounds such as Cr, MG, and GSA [3, 4]. Also, clinical therapy using rhubarb has occasionally been tried, and its

usefulness is now established [16, 17]. In particular, its effects on MG, a strong uremic toxin, were conspicuous, suggesting the presence of tannin(s). Nishioka [2] isolated more than 50 different tannins from rhubarb. In the present study, 5 different tannins characterized by their high yields were used, because large samples were necessary in order to examine their effects in rats after oral administration. A decrease in urea nitrogen, MG, and GSA was found after administration of 2.5, 5, or 10 mg of (−)-epicatechin 3-O-gallate and a lower dose (6.25 mg) of procyanidin B-2 3,3′-di-O-gallate in the same manner as after administration of a rhubarb aqueous extract. A decrease in Cr, comparable to that observed after administration of 270 mg rhubarb aqueous extract, was found in rats given 5 and 10 mg of (−)-epicatechin 3-O-gallate. In addition, whereas 6.25 mg of procyanidin B-2 3,3′-di-O-gallate caused a decrease in urea nitrogen, MG, and GSA, a 2-fold higher dose (12.5 mg) resulted in no such decrease. A lower dose (3.125 mg) of procyanidin C-1 3,3′,3″-tri-O-gallate showed no effects on blood components, whereas a higher dose, 12.5 mg, resulted in a marked increase in urea nitrogen and Cr, and a tendency of MG and GSA to increase, suggesting the aggravation of uremia. The molecular weights of these tannins were as follows: (−)-epicatechin 3-O-gallate, 442; procyanidin B-2 3,3′-di-O-gallate, 882; procyanidin C-1 3,3′,3″-tri-O-gallate, 1,322. All of these are composed of (−)-epicatechin units having a gallate moiety at position 3. (−)-Epicatechin 3-O-gallate is a monomer, procyanidin B-2 3,3′-di-O-gallate is a dimer, and procyanidin C-1 3,3′,3″-tri-O-gallate is a trimer. The uremic-toxin-decreasing action diminished with increased molecular weight. On the other hand, RG-tannin (mean molecular weight, 2,780) at a dose of 7.5 or 15 mg did not cause a decrease in any of the uremic toxins except GSA. Rhatannin, having a higher mean molecular weight (4,500), was found to cause no decrease in uremic toxins, and in fact, these toxins were increased slightly. RG-tannin is a tannin with a molecule composed of (−)-epicatechin, (−)-epicatechin 3-O-gallate, and (+)-catechin. In this molecule, 4β→8 and 4β→6 binding patterns are present in a ratio of 6:1. Rhatannin is a polymer composed of about 10 base units of (−)-epicatechin, all of the 3-positions being galloylated or with a (+)-catechin ending unit. Although we assumed, on the basis of the effects of the monomer, dimer, and trimer, that uremia would be aggravated, the results of experiments with these tannins were different from those expected. There seemed to be no rules for the biological activities of these five tannins. The monomer was found to mainly contribute to the antiuremic action of the rhubarb aqueous extract, the dimer being partially contributory. An antiuremic effect was found after administration of the rhubarb aqueous extract at a dose of 270 mg/kg body weight/day [3], and such an effect was caused by the monomer at a dose of 5 mg/kg body weight/day, demonstrating that the monomer is one of the active ingredients of rhubarb. On the other hand, (−)-epigallocatechin 3-O-gallate purified from rhubarb aqueous extract, which is different from the monomer only in that it has an OH group at each of positions 3, 4, and 5 of the B ring, had no effect on uremic toxins (data not shown), suggesting the chemical structure responsible for the manifestation of the activity. All tannins used in the present study were of the condensed type. To our knowledge, there have been no reports documenting that tannins of this type reverse the increased blood levels of urea nitrogen, Cr, MG, and GSA in renal failure. On the other hand, MG, which is currently considered to be a stronger uremic toxin, was also significantly decreased in rats given the dimer. We have demonstrated previously in in vivo and in vitro studies that creatol, a newly found Cr oxide, is produced from Cr with hydroxyl radicals and further converted into MG [18–20], thus showing the presence of an oxidizing route different from the one proposed by Aoyagi et al. [21]. Furthermore, creatol was administered intraperitoneally to normal rats, and time course changes in MG excretion were compared. The amount of MG excreted into urine during a 12-hour period after creatol administration was 210 times higher than the corresponding value in rats given Cr at an equal mol dose [18]. From these results, it is suggested that the conversion of Cr to creatol depends mainly on hydroxyl radicals, and that creatol is converted easily to MG. The elimination of activated oxygen may be involved in the tannin-induced decrease in MG. We intend to carry out further detailed investigations on this aspect.

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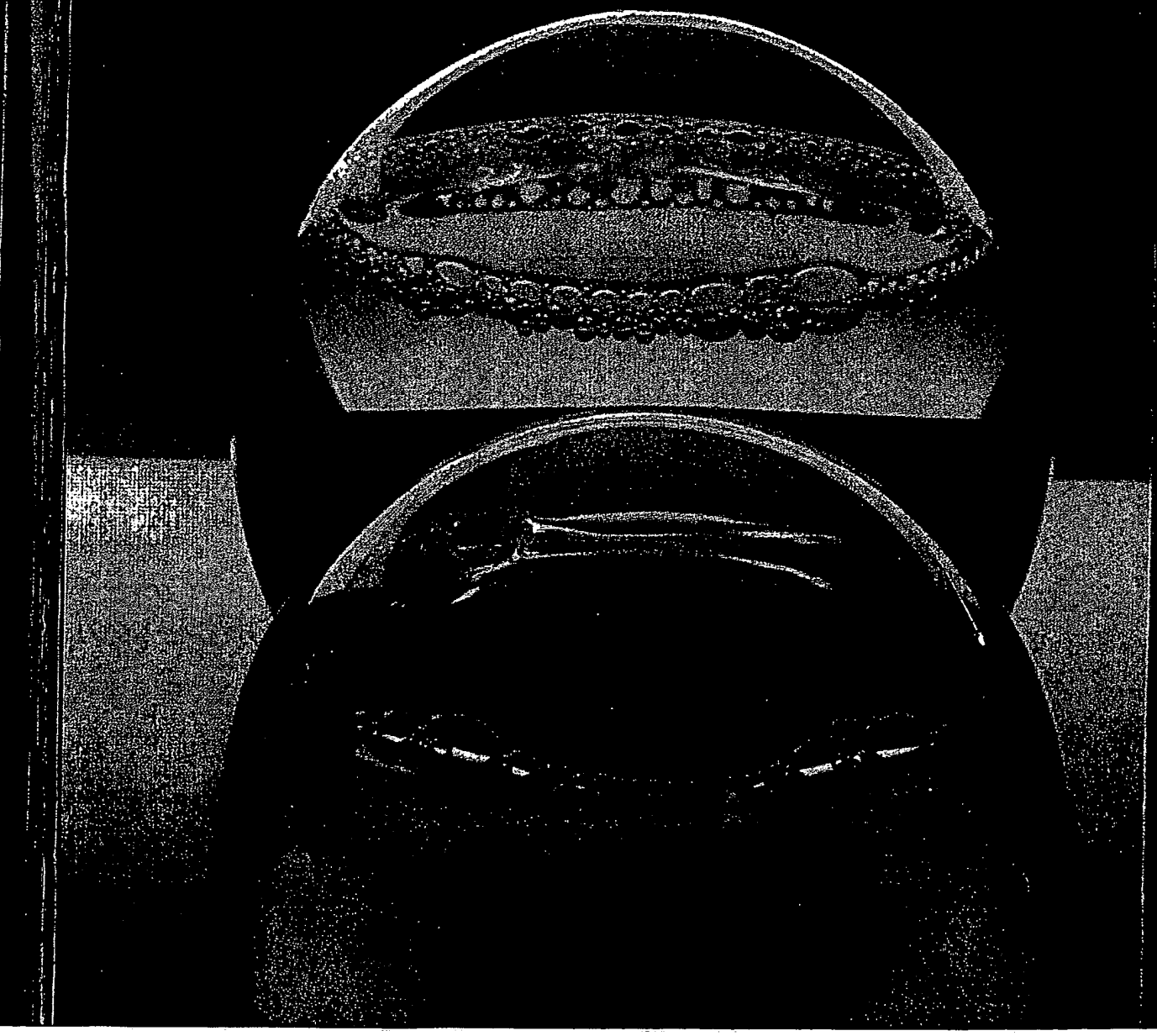
McMURRY

3

ORGANIC CHEMISTRY



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21.4 Nucleophilic Acyl Substitution Reactions of Carboxylic Acids

The most important reactions of carboxylic acids are those that convert the carboxyl group into other acid derivatives by nucleophilic acyl substitution, RCOOH → RCOY. Acid chlorides, anhydrides, esters, and amides can all be prepared from carboxylic acids (Figure 21.5).

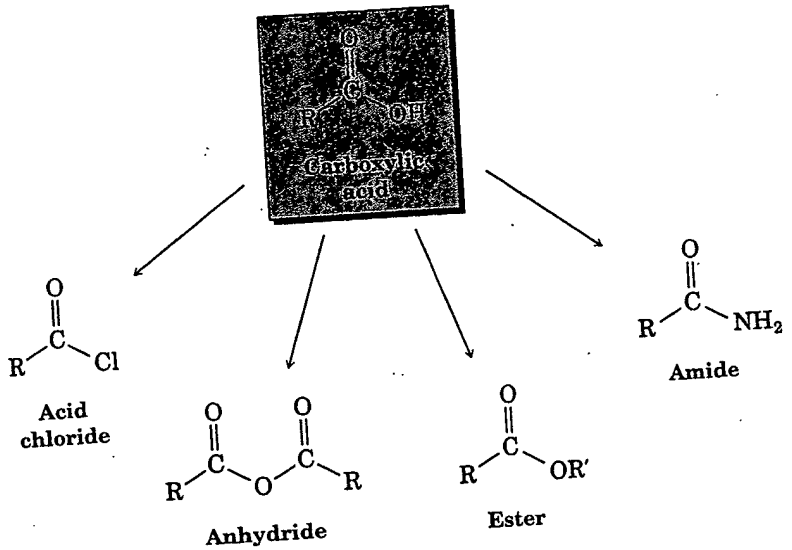


Figure 21.5 Some nucleophilic acyl substitution reactions of carboxylic acids.

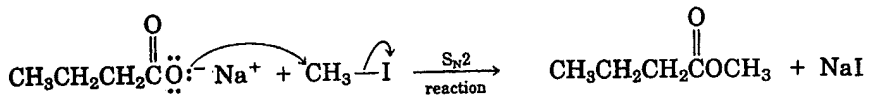
CONVERSION INTO ACID CHLORIDES

Carboxylic acids are converted into carboxylic acid chlorides by treatment with thionyl chloride ($SOCl_2$), phosphorus trichloride (PCl_3), or oxalyl chloride (ClCOCOCl). Thionyl chloride is both inexpensive and convenient to use but is strongly acidic; only acid-stable molecules can survive the reaction conditions. Oxalyl chloride, on the other hand, is much more expensive but gives higher yields and reacts under milder conditions.

$$\underset{\text{Oleic acid}}{CH_3(CH_2)_7CH{=}CH(CH_2)_7COH} \xrightarrow[\text{Benzene}]{ClCOCOCl} \underset{\text{Oleyl chloride (97\%)}}{CH_3(CH_2)_7CH{=}CH(CH_2)_7CCl} + HCl + CO + CO_2$$

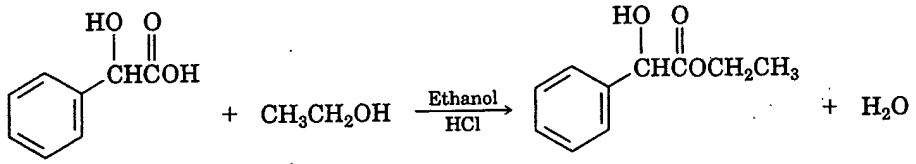
CONVERSION INTO ESTERS

One of the most important reactions of carboxylic acids is their conversion into esters, RCOOH → RCOOR′. There are many excellent methods for accomplishing this transformation, including the S_N2 reaction between a carboxylate anion nucleophile and a primary alkyl halide that we've already studied (Section 11.5).

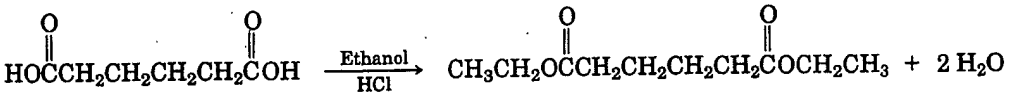


Sodium butanoate — **Methyl butanoate, an ester (97%)**

Esters can also be synthesized by a nucleophilic acyl substitution reaction between a carboxylic acid and an alcohol. Fischer[1] and Speier discovered in 1895 that esters result from simply heating a carboxylic acid in methanol or ethanol solution containing a small amount of mineral acid catalyst. Yields are good in this **Fischer esterification reaction**, but the need to use excess alcohol as solvent effectively limits the method to the synthesis of methyl, ethyl, and propyl esters.



Mandelic acid — **Ethyl mandelate (86%)**



Hexanedioic acid (adipic acid) — **Diethyl hexanedioate (95%)**

The Fischer esterification reaction, whose mechanism is shown in Figure 21.6, is a nucleophilic acyl substitution reaction carried out under acidic conditions. Although free carboxylic acids are not reactive enough to be attacked by most nucleophiles, they can be made much more reactive in the presence of a strong mineral acid such as HCl or H_2SO_4. The mineral acid acts by protonating the carbonyl-group oxygen atom, thereby giving the carboxylic acid a positive charge and rendering it much more reactive toward nucleophilic attack by alcohol. Subsequent loss of water yields the ester product.

The net effect of Fischer esterification is substitution of an –OH group by –OR′. All steps are reversible, and the reaction can be driven in either

[1]Emil Fischer (1852–1919); b. Euskirchen, Germany; Ph.D. Strasbourg (Baeyer); professor, universities of Erlangen, Würzburg, and Berlin; Nobel Prize (1902).

direction by choice of reaction conditions. Ester formation is favored when a large excess of alcohol is used as solvent, but carboxylic acid formation is favored when a large excess of water is present.

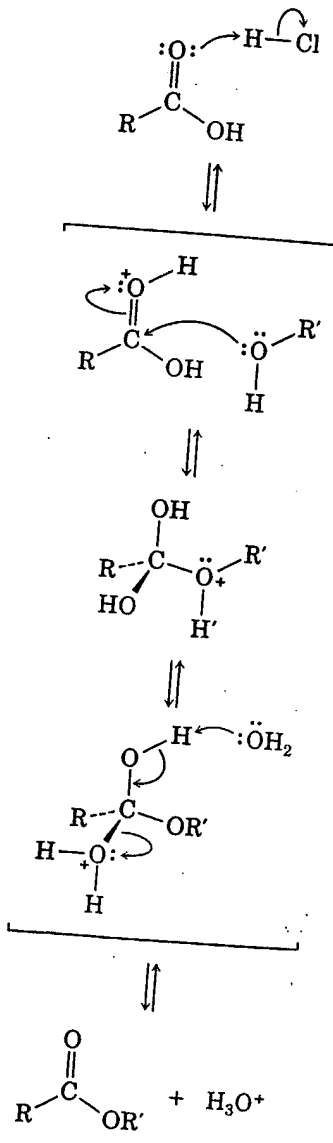
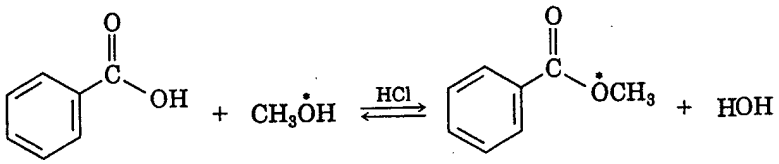


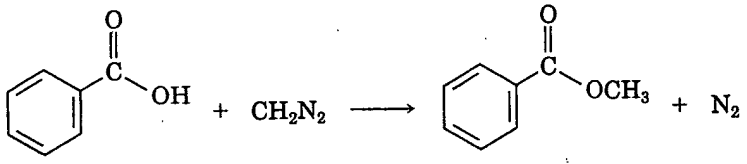
Figure 21.6 *Mechanism of Fischer esterification. The reaction is an acid-catalyzed nucleophilic acyl substitution process.*

One of the best pieces of evidence in support of the mechanism shown in Figure 21.6 comes from isotopic-labeling experiments. When ^{18}O-labeled methanol reacts with benzoic acid under Fischer esterification conditions, the methyl benzoate produced is found to be ^{18}O labeled, but the water

produced is unlabeled. This experiment shows unequivocally that it is the CO–OH bond of the carboxylic acid that is cleaved, rather than the COO–H bond, and that it is the RO–H bond of the alcohol that is cleaved, rather than the R–OH bond.



A final method of ester synthesis is the reaction between a carboxylic acid and diazomethane, CH_2N_2, a reaction first described by von Pechmann[2] in 1894. The reaction takes place instantly at room temperature to give a high yield of the methyl ester. Though quite useful, this process does not involve a nucleophilic acyl substitution reaction, since it is the COO–H bond of the carboxylic acid that is broken rather than the CO–OH bond.



Benzoic acid **Diazomethane** **Methyl benzoate (100%)**

The diazomethane method of ester synthesis is ideal, since it occurs under mild, neutral conditions and gives nitrogen gas as the only by-product. Unfortunately, diazomethane is both toxic and explosive and should be handled only in small amounts by skilled persons.

PROBLEM

21.5 Show how you would prepare these esters:
(a) Butyl acetate (b) Methyl butanoate

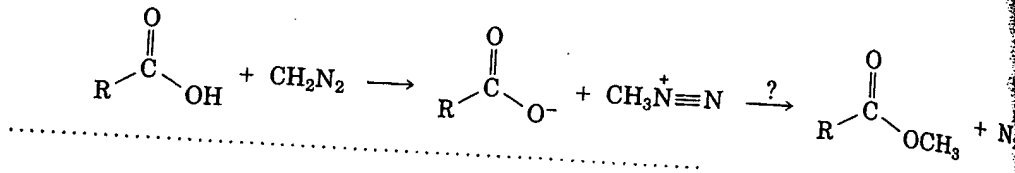
PROBLEM

21.6 If 5-hydroxypentanoic acid is treated with acid catalyst, an intramolecular esterification reaction occurs. What is the structure of the product? (*Intramolecular* means within the same molecule.)

PROBLEM

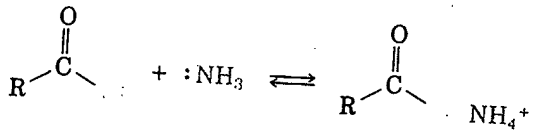
21.7 The first step in the reaction of a carboxylic acid with diazomethane is a proton transfer to give the methyldiazonium cation ($CH_3N_2^+$) and carboxylate anion. What kind of mechanism accounts for the second step?

[2]Hans von Pechmann (1850–1902); b. Nuremberg; Ph.D. Greiswald; professor, universities of Munich and Tübingen.



CONVERSION INTO AMIDES

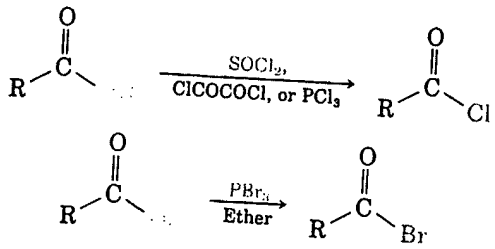
Amides are carboxylic acid derivatives in which the acid hydroxyl group has been replaced by a nitrogen substituent, $-NH_2$, $-NHR$, or $-NR_2$. Amides are difficult to prepare directly from reaction between amines and carboxylic acids, because amines are bases that convert acidic carboxyl groups into their carboxylate anions. Since the carboxylate anion has a negative charge, it is no longer electrophilic and no longer likely to be attacked by nucleophiles.



21.5 Chemistry of Acid Halides

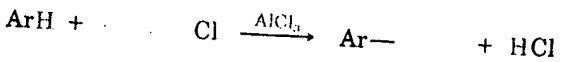
PREPARATION OF ACID HALIDES

Acid chlorides are prepared from carboxylic acids by reaction with thionyl chloride ($SOCl_2$), oxalyl chloride (ClCOCOCl), or phosphorus trichloride (PCl_3), as we saw in the previous section. Reaction of an acid with phosphorus tribromide (PBr_3) yields the acid bromide.



REACTIONS OF ACID HALIDES

Acid halides are among the most reactive of carboxylic acid derivatives and can be converted into a variety of other kinds of compounds. For example, we've already seen the value of acid chlorides in preparing aryl alkyl ketones via the Friedel–Crafts reaction (Section 16.4).



Most acid halide reactions occur by a nucleophilic acyl substitution mechanism. As illustrated in Figure 21.7, the halogen can be replaced by –OH to yield an acid, by –OR to yield an ester, or by $-NH_2$ to yield an amide. In addition, the reduction of acid halides yields primary alcohols, and reaction with Grignard reagents yields tertiary alcohols. Although the reactions in Figure 21.7 are illustrated only for acid chlorides, they also take place with other acid halides.

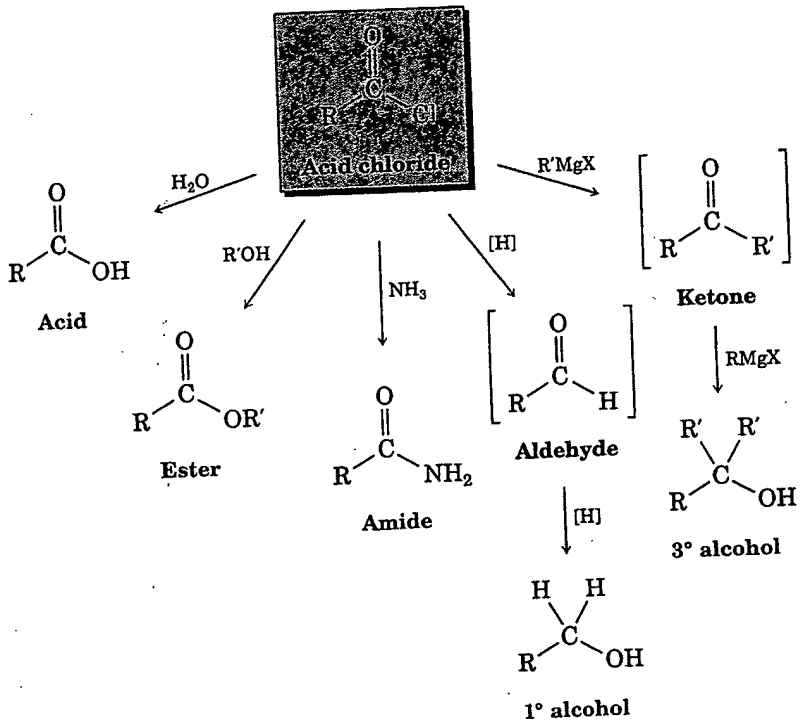
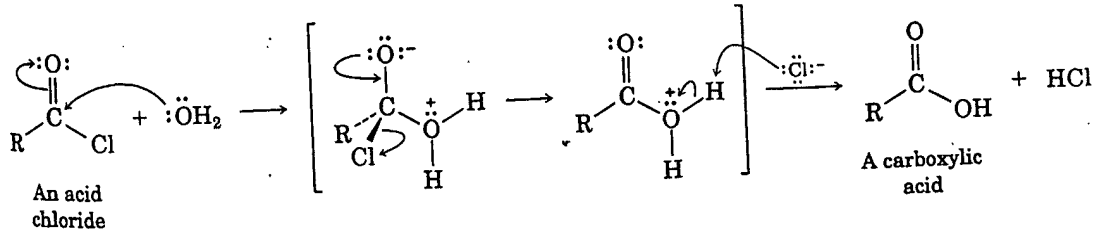


Figure 21.7 Some nucleophilic acyl substitution reactions of acid chlorides.

Hydrolysis: Conversion of Acid Halides into Acids Acid chlorides react with water to yield carboxylic acids. This hydrolysis reaction is a typical nucleophilic acyl substitution process initiated by attack of water on the acid chloride carbonyl group. The initially formed tetrahedral intermediate undergoes elimination of chloride ion and loss of a proton to give the product carboxylic acid plus HCl.



Glycosylated Derivatives of Benzophenone, Benzhydrol, and Benzhydril as Potential Venous Antithrombotic Agents

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A series of glycosylated derivatives of benzophenone, benzhydrol, and benzhydril has been synthesized and evaluated for potential activity as venous antithrombotic agents. Studies on structure–activity relationships revealed that compounds having an electron-withdrawing group in the benzhydril or benzhydrol moiety, and specifically those having the β-D-xylopyranosyl structure in the sugar moiety, were good antithrombotic agents in a rat model of venous thrombosis.

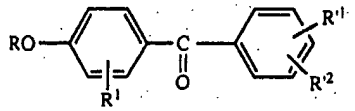
Introduction

Heparin, a glycosaminoglycan component of a parent proteoglycan, is a highly sulfated polydisperse polysaccharide that has been used for over half a century as an anticoagulant and antithrombotic agent.[1] Heparin exhibits the undesirable side effect of hemorrhagic complications after intravenous injection.[1a,2] Thus, although heparin enjoys widespread clinical use, it has been cited as the drug most responsible for death in otherwise healthy patients.[3]

The relatively recent introduction of low-molecular-weight (LMW) fractions of heparin obtained by various means (fractionation, depolymerization) has provided therapeutic agents offering a supposedly decreased risk of hemorrhage.[4] Fractions having a molecular weight of less than 5000 retain their ability to bind to antithrombin III (AT III) and to inhibit activated factor X (Xa), but the antithrombin activity is greatly decreased.[5] Furthermore, the therapeutic potential of these compounds is improved because of their more favorable bioavailability, even after subcutaneous administration.[6] Dermatan sulfate (DS), a heterogeneous glycosaminoglycan that is also a component of a parent proteoglycan, had been shown experimentally to be antithrombotic, even though it is devoid of AT III-binding activity, the compound inhibiting thrombin via heparin co-factor II (HC II).[7] Thus, although this compound elicits a somewhat less potent antithrombotic effect, the risk of hemorrhage is virtually absent.[8] However DS has to be obtained by tedious extraction and is only effective when administrated parenterally.[9] Although DS is absorbed intact, its bioavailability is low, and therapeutically active blood levels are rarely obtained.[10]

This paper describes for the first time the venous antithrombotic effect of a chemically defined entities following intraperitoneal, oral, and intravenous administration in the rat.

Chemistry: see Scheme I. As a part of broader study on venous antithrombotic agents, a number of novel glycosylated benzophenone derivatives having the formula I were selected for general screening. Various methods for producing glycosides in a highly stereoselective manner have been reported.[11] In the present work, the classical Koenigs–Knorr reaction[11] employing Ag_2O as activator



R = glycosyl

R^1, R'^1, R'^2 = H, alkyl, halo, NO_2, CF_3, CN, OMe

was the most commonly used (method B). In some cases, NaH was selected as the base for the glycosidation reaction (method A). The 1,2-cis glycosides 11 and 12 were prepared from the per-*O*-acetylglycopyranose together with a Lewis acid such as $SnCl_4$ as activator for the glycosidation[12] (method C).

The target benzhydrol (V) and benzhydril glycosides (VI) were prepared from the appropriate protected glycosyl precursors and benzophenone derivatives (I).[13] The benzophenone phenols were obtained by condensation of the Grignard reagent of the appropriate substituted anisoles with an aromatic acyl chloride[14] (except for the nitro derivative of benzophenone), followed by demethylation in acid medium of the resultant methoxy ether. The 4′-nitro-3-methoxybenzophenone was obtained by the reaction of 4-fluoro-1-nitrobenzene and a substituted morpholine.[15] The latter was synthesized from 3-methoxybenzaldehyde, morpholine, and potassium cyanide in acid medium.[15] The target benzophenone phenols were obtained in excellent yields, and their physical constants are summarized in Table I.

The methods of synthesis and data for the glycosylated products are summarized in Table II.

Two routes were chosen to obtain the benzhydril glycosides (VI). First, the benzophenone β-D-xylopyranosides (II) were reduced with sodium borohydride in trifluoroacetic acid[16] with subsequent deacylation by the Zemplén method,[17] affording compounds VI in good to excellent yield. Alternatively, the β-D-xylopyranosides (IV) were deacylated and then reduced to the benzhydrols (V) with sodium borohydride in methanol to give the latter as mixtures of epimers without induction of reduction in excellent yield.[18] The benzhydrils (VI) were then obtained by reduction of the benzhydrols (V) in the manner used for reduction of the benzophenones to the benzhydril.

Pharmacology Evaluation

1. **Technique.** The technique employed involves partial stasis in the presence of slight endothelium

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Scheme I

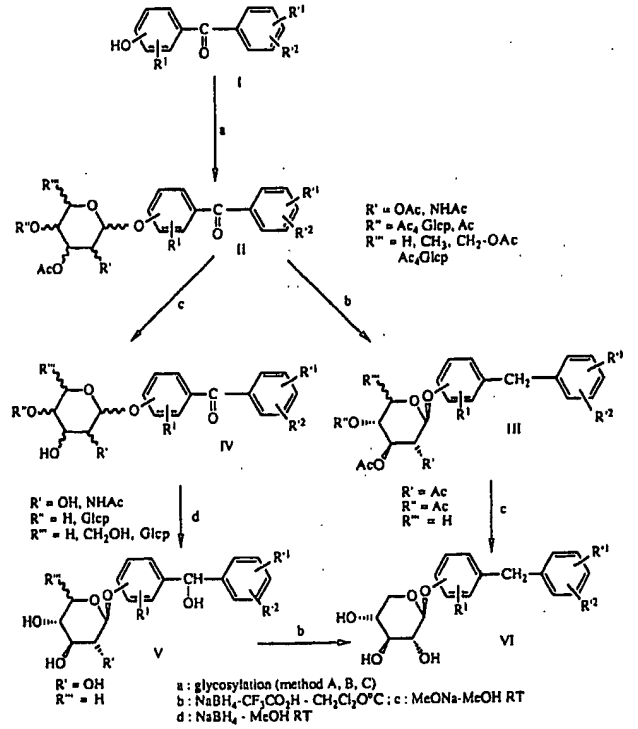
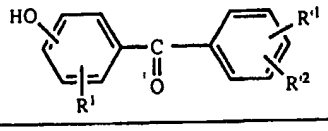


Table I



position of OH	R'1	R'2	R1	mp, °C
4	2'-CH_3	H	3-CH_3	142
3	4'-NO_2	H	H	117
3	2'-CH_3	H	H	112
2	2'-CH_3	H	5-CH_3	95

alteration, as previously described by Millet et al.[19] Male Wistar rats (IFFA CREDO, France) weighing 250 g are anaesthetized with urethane (10 mL/kg body weight of a 12% urethane solution by intraperitoneal injection). A midline laparotomy is performed, and abdominal organs are displayed on saline-impregnated gauze. The inferior vena cava is cleared from surrounding tissues from the left renal vein to the iliac vein. A partial ligature just beneath the left renal vein is made with a cotton thread enclosing the vein together with a G 26 3/8-in. gauge needle. A Schwartz clamp is placed just above the iliac bifurcation to delimit a venous bag.

Saline is flushed into this bag at a constant rate of 10 mL/min during 15 s at a point just above the clamp. Thereafter, the perfusion needle is removed and the hole sealed with a cyanoacrylate adhesive, after which the needle and the clamp are removed. Organs are replaced, and the body temperature of the rats was maintained at a temperature close to 37 °C. Fifteen minutes after the partial venous flow has been restored, two Schwartz clamps are placed, one at the level of the structure and the other at the iliac bifurcation. The vein segment is split longitudinally, and the thrombus is removed and dried for 24 h at 55 °C before weighing.

The activity of these compounds is given as percentage reduction of thrombus formation by weight.

2. **Treatment.** The compounds were administered intraperitoneally to rats with (carboxymethyl) cellulose as vehicle at doses of 100 mg/kg in a volume of 5 mL/kg for the substances of which the lethal dose exceeded 800 mg/kg (in mice). Otherwise antithrombotic activity was assessed at $^1/_{10}$ of the LD_{50}.

The heparin used as reference compound was monitored by iv route at various time intervals before thrombogenic challenge with factor Xa, a dose-related antithrombotic effect, this being significant from the dose 0.12 mg/kg and producing an ED_{50} (dose inhibiting the effect of factor Xa by 50%) of 0.15 mg/kg.

Results and Discussion

As may be seen from Table III, significant antithrombotic activity, although admittedly low by the intraperitoneal route, was displayed solely by the D-xylopyranosides having the β configuration at the anomeric carbon atom. Other sugar structures and configurations were inactive. The results shown in Table III prompted the synthesis of a number of β-D-xylopyranosides of benzophenone derivatives for systematic evaluation of structure–activity relationships. The antithrombotic activity of these compounds is summarized in Table IV.

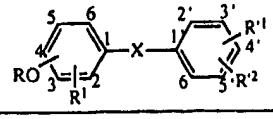
The data of Table IV showed that compound 70, having the β-D-xylopyranosyl group at the meta position of 4'-nitrobenzophenone, was more active than the para-substituted analog (compound 55). The analog of 70 lacking the 4'-nitro group (compound 74) was about as active as 70. No clear structure–activity trends toward antithrombotic activity could be discerned from compounds 60 and 68 having an electron-donating group at C-4' and compounds 70 and 56 where the 4'-substituent is electron-withdrawing.

Some of the benzophenone derivatives in Table IV were selected for reduction of the carbonyl group to the corresponding benzhydrol and/or benzhydril compounds. The antithrombotic activities of these products are summarized in Table V. Surprisingly the reduction of the para-glycosylated 4'-nitrobenzophenone 55 (itself inactive as a venous antithrombotic compound) afforded the highly active benzhydrol 79 and the benzhydril 86. These two products are the leading candidates for effective antithrombotic agents in the rat model. The compounds 79 and 86 have been selected for evaluation of their oral antithrombotic activity in the rat model.[19] The technique employed was based on that of Wessler as described in the rabbit.[20] Oral administration of these two compounds was 4 h before injection of the thrombogenic stimulus (Xa) and reduced thrombus weight in a dose-related manner with an ED_{50} of 48 mg/kg of body weight for 79 and an ED_{50} of 24 mg/kg of body weight for 86.

Up to now, heparin has not yet been revealed to be active by oral administration. Compound 79, being more soluble in the poly(ethylene glycol) 400, was chosen for comparison with heparin by intravenous route. The data are summarized in Table VI.

Conclusion and Perspective in the Future. We have reported for the first time the synthesis of the β-D-xylopyranoside and demonstrated a venous antithrombotic activity following oral administration (79, 86). The biological activity of the separated epimers of 79 (mixture 50/50) is quite the same as the parent compound (unpublished results). We believe that β-D-xylopyranose was the pharmacophore for the antithrombotic activity. How-

Table II



no.	position	R	R'1	R'2	R1	mp, °C	formula	anal.	X	$[\alpha]^{20}_D$, deg	method	procedure	yield, %
1	4	Ac4-β-Glcp	4'-NO2	H	H	214	$C_{27}H_{27}NO_{13}$	CHN	C=O	−88 (c 0.5, $CHCl_3$)	B		37
2	4	Ac4-β-Glcp	4'-Cl	H	H	212	$C_{27}H_{27}ClO_{11}$	CH	C=O	−20.5 (c 0.5, $CHCl_3$)	B		71
3	4	Ac4-β-Glcp	H	H	H	170	$C_{27}H_{28}O_{11}$	CH	C=O	−21.2 (c 0.5, $CHCl_3$)	B		81
4	4	Ac4-β-Galp	4'-NO2	H	H	148	$C_{27}H_{27}NO_{13}$	CHN	C=O	+3.2 (c 0.5, $CHCl_3$)	B		61
5	4	Ac4-β-Galp	4'-Cl	H	H	140	$C_{27}H_{27}ClO_{11}$	CH	C=O	+5.6 (c 0.5, $CHCl_3$)	B		45
6	4	Ac3-β-GlcNAcp	4'-NO2	H	H	238	$C_{27}H_{28}N_2O_{12}$	CHN	C=O	−15.3 (c 0.5, $CHCl_3$)	A		46
7	4	Ac3-β-GlcNAcp	2'-Cl	H	H	196	$C_{27}H_{28}NClO_{10}$	CHN	C=O	−10.2 (c 0.5, $CHCl_3$)	A		60
8	4	Ac3-β-GlcNAcp	4'-CF_3	H	H	200	$C_{28}H_{28}NF_3O_{10}$	CHN	C=O	−14.8 (c 0.5, $CHCl_3$)	A		56
9	4	Ac3-β-GlcNAcp	2'-CH_3	H	3-CH_3	175	$C_{29}H_{31}NO_{10}$	CHN	C=O	−16.9 (c 0.5, $CHCl_3$)	A		51
10	4	Ac4-α-Manp	4'-NO2	H	H	70 foam	$C_{27}H_{27}NO_{13}$	CHN	C=O	+78 (c 0.5, $CHCl_3$)	B		29
11	4	Ac4-β-Manp	4'-NO2	H	H	120	$C_{27}H_{27}NO_{13}$	CHN	C=O	−73.3 (c 0.5, $CHCl_3$)	C		50
12	4	Ac3-α-Xylp	4'-NO2	H	H	foam	direct desacetylated		C=O	+130 (c 0.5, $CHCl_3$)	C		26
13	4	Ac3-β-Xylp	4'-NO2	H	H	149	$C_{24}H_{23}NO_{11}$	CHN	C=O	−33 (c 1, $Cl(CH_2)_2Cl$)	B		65
14	4	Ac3-β-Xylp	4'-CN	H	H	150	$C_{25}H_{23}NO_9$	CHN	C=O	−29 (c 0.5, $CHCl_3$)	B		55
15	4	Ac3-β-Xylp	4'-Cl	H	H	148	$C_{24}H_{23}ClO_9$	CH	C=O	−44 (c 1, $CHCl_3$)	B		72
16	4	Ac3-β-Xylp	2'-CH_3	H	3-CH_3	102	$C_{26}H_{28}O_9$	CH	C=O	−33 (c 0.5, MeOH)	B		50
17	4	Ac3-β-Xylp	4'-CF_3	H	H	134	$C_{25}H_{23}F_3O_9$	CH	C=O	NA	B		28
18	4	Ac3-β-Xylp	4'-OCH_3	H	H	144	$C_{25}H_{26}O_{10}$	CH	C=O	−21 (c 0.5, MeOH)	B		20
19	4	Ac3-β-Xylp	H	H	H	132	$C_{24}H_{24}O_9$	CH	C=O	−44 (c 0.5, $CHCl_3$)	B		30
20	4	Ac7-β-Mal[c]	4'-NO2	H	H	191	$C_{39}H_{43}NO_{21}$	CHN	C=O	+34 (c 0.5, $CHCl_3$)	B		48
21	4	Ac7-β-Lac[d]	4'-NO2	H	H	100	$C_{39}H_{43}NO_{21}$	CHN	C=O	−26 (c 0.5, $CHCl_3$)	B		40
22	4	Ac3-β-GlcpA[b]	4'-NO2	H	H	NA	$C_{26}H_{25}NO_{13}$	CHN	C=O	NA	B		56
23	4	Ac3-α-Rhap	4'-NO2	H	H	foam	$C_{25}H_{25}NO_{11}$	CHN	C=O	−85 (c 0.5, $CHCl_3$)	B		43
24	4	Ac3-β-Xylp	3'-NO2	H	H	145	$C_{24}H_{23}NO_{11}$	CHN	C=O	−26 (c 0.5, MeOH)	B		75
25	4	Ac3-β-Xylp	2'-Cl	4'-Cl	H	142	$C_{25}H_{22}Cl_2O_9$	CH	C=O	−20 (c 0.5, MeOH)	B		85
26	4	Ac3-β-Xylp	4'-CH_3	H	H	138	$C_{25}H_{26}O_9$	CH	C=O	−22 (c 0.5, MeOH)	B		60
27	4	Ac3-β-Xylp	2'-Cl	H	H	165	$C_{24}H_{23}ClO_9$	CH	C=O	−24 (c 0.5, MeOH)	B		59
28	3	Ac3-β-Xylp	4'-NO2	H	H	144	$C_{24}H_{23}NO_{11}$	CHN	C=O	NA	B		73
29	3	Ac3-β-Xylp	4'-Cl	H	H	126	$C_{24}H_{23}ClO_9$	CH	C=O	−26 (c 0.5, MeOH)	B		53
30	3	Ac3-β-Xylp	2'-CH_3	H	H	90	$C_{25}H_{26}O_9$	CH	C=O	−40 (c 0.5, $CHCl_3$)	B		71
31	3	Ac3-β-Xylp	4'-CH_3	H	H	94	$C_{25}H_{26}O_9$	CH	C=O	−45 (c 0.5, $CHCl_3$)	B		80
32	3	Ac3-β-Xylp	H	H	H	148	$C_{24}H_{24}O_9$	CH	C=O	−28 (c 0.5, MeOH)	B		72
33	2	Ac3-β-Xylp	2'-CH_3	H	5'-CH_3	NA	$C_{26}H_{28}O_9$	CH	C=O	NA	B		
34	4	Ac3-β-Xylp	4'-OCH_3	H	H	80	$C_{25}H_{28}O_9$	CH	CH_2	−21 (c 0.5, EtOAc)		A	62
35	4	Ac3-β-Xylp	H	H	H	112	$C_{24}H_{26}O_8$	CH	CH_2	−27 (c 1, EtOAc)		A	42
36	4	Ac3-β-Xylp	3'-NO2	H	H	105	$C_{24}H_{25}NO_{10}$	CHN	CH_2	−25 (c 0.5, EtOAC)		A	43
37	4	Ac3-β-Xylp	4'-NO2	H	H	foam	$C_{24}H_{25}NO_{10}$	CHN	CH_2	−33 (c 0.7, $CHCl_3$)		A	62
38	4	Ac3-β-Xylp	2'-Cl	H	H	85	$C_{24}H_{25}ClO_8$	CH	CH_2	−21 (c 0.5, $CHCl_3$)		A	43
39	4	Ac3-β-Xylp	4'-Cl	H	H	101	$C_{24}H_{25}ClO_8$	CH	CH_2	−19 (c 0.5, AcOEt)		A	39
40	4	Ac3-β-Xylp	2'-Cl	4'-Cl	H	99	$C_{24}H_{24}Cl_2O_8$	CH	CH_2	−17 (c 0.5, EtOAC)		A	65
41	3	Ac3-β-Xylp	4'-Cl	H	H	99	$C_{24}H_{25}ClO_8$	CH	CH_2	−43 (c 0.5, $CHCl_3$)		A	100
42	3	Ac3-β-Xylp	H	H	H	110	$C_{24}H_{26}O_8$	CH	CH_2	−46 (c 0.5, $CHCl_3$)		A	100

[a] All sugars are of the D configuration, except for rhamnose, which is L. [b] As methyl ester. [c] Hepta-O-acetyl-β-maltosyl. [d] Hepta-O-acetyl-β-lactosyl. NA: not available. The $J_{1,2}$ values in ^{1}H-NMR spectra for the component sugar were all in range 7–9 Hz, except for compounds 11 and 12 (2–4 Hz).[24]

ever, the dose used was high. Our target in the future is thus active compounds at low doses by changing the heteroatom of D-xyloside series. Of course, the mechanism of action will be investigated in our laboratory.

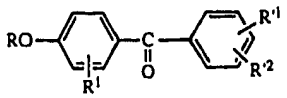
Experimental Section

Melting points were determined on a Kofler melting-point apparatus. ^{1}H-NMR spectra were recorded for $CDCl_3$ or Me_2SO-d_6 solutions with a Brucker WD 80 FT instrument. Optical rotations were recorded with a Perkin-Elmer Model 241 or a Jobin Yvon digital-readout polarimeter. Satisfactory elemental analyses for C, H, N were obtained for all new compounds.

Method A. 4-(4'-Nitrobenzoyl)phenyl 2-Acetamido-3,4,5-tri-O-acetyl-2-deoxy-β-D-glucopyranoside (7). To a solution of 5 g (20.5 mmol) of (4'-nitrobenzoyl)phenol in DMF (25 mL) and dichloromethane (25 mL) was added 0.54 g (22 mmol) of NaH under a nitrogen atmosphere. The mixture was stirred for 2 h at room temperature, and then 8.3 g (23.25 mmol) of 2-acetamido-3,4,6-tri-O-acetyl-2-deoxy-α-D-glucopyranosyl chloride[21] was added portionwise and the final solution was stirred for 3 h at 40 °C. The mixture was poured into ice–water, and then the aqueous solution was extracted with ethyl acetate. The organic layer was washed with 1 M NaOH and with water until the pH was neutral and dried over $MgSO_4$. Ethyl acetate was removed by evaporation under diminished pressure. The residual oil was treated with ethyl ether and the resultant precipitate recrystallized from ethyl acetate to yield compound 7 (6.9 g, 46%): mp 238 °C; $[\alpha]^{20}_D$ −15.3° (c 0.5, $CHCl_3$); ^{1}H NMR (Me_2SO-d_6) δ 1.79 (s, 3 H, NHAc), 1.95 (s, 3 H, OAc), 2.00 (s, 6 H, OAc) 4.17 (m, 4 H), 4.98 (m, 1 H), 5.22 (dd, J = 9.5 Hz, 1 H) 5.58 (d, $J_{1,2}$ = 8.35 Hz, 1 H, H-1), 7.2 (d, J = 8.4 Hz, 2 H, Ar), 7.8 (d, 2 H Ar), 7.93 (d, 2 H, Ar), 8.10 (d, J = 9.6 Hz, 1 H, NH), 8.40 (d, 2 H, Ar).

Method B. [4-(4-Nitrobenzoyl)phenyl]2,3,4-tri-O-acetyl-β-D-xylopyranoside (14). A mixture of (4-nitrobenzoyl)phenol (2.45 g, 10 mmol) and 2,3,4-tri-O-acetyl-α-D-xylopyranosyl bromide[22] (3.4 g, 10 mmol) in anhydrous CH_3CN (200 mL) was stirred with 2.4 g (10.3 mmol) of freshly prepared Ag_2O for 30 min at ambient temperature. Ethyl acetate (500 mL) was added, and the solid salt was removed by filtration. The organic layer was washed with 1 M NaOH and water until the pH was neutral and then dried over $MgSO_4$. After evaporation under diminished pressure, the residual oil was crystallized from ethyl ether and

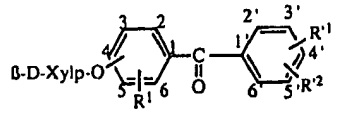
Table III



no.	R[a]	R'1	R'2	R1	mp, °C	formula	anal.	$[\alpha]^{20}_D$, deg	LD_0	activity,[d] %
43	β-Glcp	4'-NO_2	H	H	196	$C_{19}H_{19}NO_9 \cdot H_2O$	CHN	−54 (c 0.5, MeOH)	>800	0
44	β-Glcp	4'-Cl	H	H	155	$C_{19}H_{19}ClO_7 \cdot {}^1/_2H_2O$	CHCl	−57 (c 0.5, MeOH)	>800	0
45	β-Glcp	H	H	H	163	$C_{19}H_{20}O_7 \cdot {}^1/_2H_2O$	CH	−55 (c 0.5, MeOH)	>800	25 (NS)
46	β-Galp	4'-NO_2	H	H	220	$C_{19}H_{19}NO_9$	CHN	−39 (c 0.6, Py)	>800	0
47	β-Galp	4'-Cl	H	H	202	$C_{19}H_{19}ClO_7 \cdot {}^1/_2H_2O$	CHCl	−43 (c 0.5, MeOH)	>800	0
48	β-GlcpNAc	4'-NO_2	H	H	206	$C_{21}H_{22}N_2O_5 \cdot H_2O$	CHN	+12.5 (c 0.6, MeOH)	>800	0
49	β-GlcpNAc	2'-Cl	H	H	226	$C_{21}H_{22}NO_7$	CHNCl	+20 (c 0.4, MeOH)	>800	20 (NS)
50	β-GlcpNAc	4'-CF_3	H	H	238	$C_{22}H_{22}F_3NO_7 \cdot {}^1/_2H_2O$	CHNF	+20 (c 0.4, MeOH)	>800	19 (NS)
51	β-GlcpNAc	2'-CH_3	H	3-CH_3	238	$C_{23}H_{27}NO_7$	CHN	+4 (c 0.5 MeOH)	>800	12 (NS)
52	α-Manp	4'-NO_2	H	H	206	$C_{19}H_{19}NO_9$	CHN	+120 (c 0.5, MeOH)	>800	0
53	β-Manp	4'-NO_2	H	H	120	$C_{19}H_{19}NO_9$	CHN	−58 (c 0.5, MeOH)	>800	0
54	α-Xylp	4'-NO_2	H	H	172	$C_{18}H_{17}NO_8$	CHN	+163 (c 0.5, MeOH)	600[e]	38 (NS)
55	β-Xylp	4'-NO_2	H	H	200	$C_{18}H_{17}NO_8$	CHN	−27 (c 0.5, MeOH)	>800	0
56	β-Xylp	4'-CN	H	H	206	$C_{19}H_{17}NO_6$	CHN	−29 (c 0.3, MeOH)	ND	0
57	β-Xylp	4'-Cl	H	H	174	$C_{18}H_{17}ClO_6$	CH	−27 (c 0.1, MeOH)	>800	37
58	β-Xylp	2'-CH_3	H	3CH3	135	$C_{20}H_{22}O_6$	CH	−25 (c 0.5, MeOH)	ND	62
59	β-Xylp	4'-CF_3	H	H	160	$C_{19}H_{17}F_3O_6$	CH	−20 (c 0.6, MeOH)	>800	0
60	β-Xylp	4'-CH_3O	H	H	180	$C_{19}H_{20}O_7$	CH	−26 (c 0.6, MeOH)	>800	44
61	β-Xylp	H	H	H	140	$C_{18}H_{18}O_6$	CH	−20 (c 0.7, EtOAc)	750[e]	77
62	β-Mal[c]	4'-NO_2	H	H	158	$C_{25}H_{29}NO_{14}$	CHN	+64 (c 0.5, MeOH)	>800	17 (NS)
63	β-Lac	4'-NO_2	H	H	208	$C_{25}H_{29}NO_{14}$	CHN	−21 (c 0.5, Pyr)	>800	0
64	β-GlcpA	4'-NO_2	H	H	132	$C_{19}H_{17}NO_{10}$	CHN	−68 (c 0.5, MeOH)	>800	20 (NS)
65	α-Rhap	4'-NO_2	H	H	110	$C_{19}H_{19}NO_8$	CHN	−123 (c 0.5, MeOH)	>800	0

[a] All sugars are of the D configuration, except for rhamnose, which is L. [b] Lac = lactose. [c] Mal = maltose. [d] Antithrombotic activity at 100 mg/kg for compounds where the nonlethal dose (LD_0) >800 mg, otherwise antithrombotic activity was assessed at $^1/_{10}$ of LD_{50}; activity is given by intraperitoneal route as percentage of reduction of thrombus formation by weight, and 5–10 rats are used by lot of tests. The injection of the stimulus was 4 h after administration of the tested compound. [e] LD_{50}, ND = not determined, NS = not significant.

Table IV



no.	position of β-D-Xylp	R'1	R'2	R1	mp, °C	formula	anal.	$[\alpha]^{20}_D$, deg	LD_0	activity,[a] %
66	4	3'-NO_2	H	H	164	$C_{18}H_{17}NO_8$	CHN	−30 (c 0.2, MeOH)	>800	39
67	4	2'-Cl	4'-Cl	H	172	$C_{18}H_{16}Cl_2O_6 \cdot {}^1/_2H_2O$	CH	−22 (c 0.7, MeOH)	>800	18 (NS)
68	4	4'-CH_3	H	H	162	$C_{19}H_{20}O_6 \cdot H_2O$	CH	−27 (c 0.6, MeOH)	>800	0
69	4	2'-Cl	H	H	90	$C_{18}H_{17}ClO_6 \cdot H_2O$	CH	−21 (c 0.7, MeOH)	>800	76
70	3	4'-NO_2	H	H	108	$C_{18}H_{17}NO_8$	CHN	−30 (c 0.7, MeOH)	>800	61
71	3	4'-Cl	H	H	80	$C_{18}H_{17}ClO_6 \cdot {}^1/_2H_2O$	CHCl	−26 (c 0.5, MeOH)	220[b]	0
72	3	2'-CH_3	H	H	168	$C_{19}H_{20}O_6$	CH	−33 (c 0.8, MeOH)	>800	0
73	3	4'-Me	H	H	154	$C_{19}H_{20}O_6$	CH	−29 (c 0.8, MeOH)	>800	0
74	3	H	H	H	140	$C_{18}H_{18}O_6$	CH	−30 (c 0.5, MeOH)	>800	60
75	2	2'-Me	H	5-CH_3		$C_{18}H_{18}O_6$	CH	NA	>800	0

[a] See Table I. [b] LD_{50}. NA = not available.

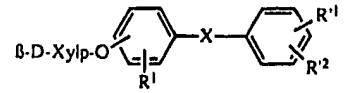
recrystallized from MeOH to yield 6.5 g (65%) of 14: mp 149 °C; $[\alpha]^{20}_D$ −33° (c 1, 1,2-dichloroethane); ^{1}H NMR (Me_2SO-d_6) δ 2.00 (s, 9 H, OAc), 3.90 (m, 2H, H-5), 5.16 (m 3 H, H-2,3,4), 5.70 (d, $J_{1,2}$ = 6.5 Hz 1 H, H-1), 7,19 (d, J = 8.0 Hz, 2 H, Ar), 7.80 (d, J = 8 Hz, 2 H, Ar), 7.90 (d, J = 8 Hz, 2 H, Ar), 8.4 (d, J = 8 Hz, 2 H, Ar).

Method C. 4-(4-Nitrobenzoyl)phenyl 2,3,4-Tri-*O*-acetyl-α-D-xylopyranoside (13). To a solution of 10 g (31.4 mmol) of 1,2,3,4-tetra-*O*-acetyl-β-D-xylopyranose[23] and 10 g (41 mmol) of a (4-nitrobenzoyl)phenol solution in CH_2Cl_2 (100 mL) was added $SnCl_4$ (8 mL, 68 mmol) under a nitrogen atmosphere. The mixture was stirred for 4 h at 60 °C and then poured onto ice. The residue was extracted with CH_2Cl_2, washed with a solution of $NaHCO_3$ and water until the pH was neutral, and then dried over $MgSO_4$. Evaporation under diminished pressure gave an oil that was chromatographed on a column of silica gel (solvent 8:1 $CHCl_3$–EtOAc). The solvent was removed to afford 13 as an oil yield 6 g (26%): $[\alpha]^{20}_D$ +130° (c 0.5, $CHCl_3$); ^{1}H NMR (Me_2SO-d_6) δ 2.00 (s, 3 H, OAc), 2.04 (s, 6 H, OAc), 3.70 (m, 2 H), 5.14 (m, 2 H), 5.55 (t, 1 H), 6.03 (d, $J_{1,2}$ = 3.5 Hz, 1 H, H-1), 7.30 (d, J = 8 Hz, 2 H, Ar), 7.80 (d, J = 8 Hz, 2 H Ar), 7.90 (d, J = 8 Hz, 2 H, Ar), 8.4 (d, J = 8 Hz, 2 H, Ar).

Reduction of Benzophenone to Benzhydril (Procedure A). 4-(4-Nitrobenzyl)phenyl 2,3,4-Tri-*O*-acetyl-β-D-xylopyranoside (38). To a mixture of 2.45 g (5 mmol) of 14 in CF_3CO_2H (15 mL) and CH_2Cl_2 (20 mL) was added portionwise at 0 °C 1.17 g (30 mmol) of $NaBH_4$. The mixture was stirred at room temperature for 8 h until the starting material disappeared (solvent 4:1 toluene–EtOAc). The residue was hydrolyzed on ice, and the acid was neutralized with a solution of $NaHCO_3$. The aqueous phase was then extracted with EtOAc and the extract washed with water until it was neutral. Evaporation under diminished pressure gave an oil that was chromatographed on a column of silica gel with 9:1 toluene–EtOAc to yield 5.5 g (100%) of 38 as an oil: $[\alpha]^{20}_D$ −33° (c 0.5, $CHCl_3$); ^{1}H NMR ($CDCl_3$) δ 2.07 (3 H, OAc), 2.08 (3 H, OAc), 2.14 (3 H, OAc), 3.70 (dd, J = 2.6 Hz, J = 13 Hz, 1 H, H-5), 4.02 (s, 2 H), 4.12 (dd, J = 4 Hz, J = 13 Hz, 1 H, H-5'), 5.07 (d, $J_{1,2}$ = 7 Hz, 1 H, H-1), 5.34 (m, 3 H), 6.94 (d, J = 9 Hz, 2 H, Ar), 7.11 (d, J = 9 Hz, 2 H, Ar, NaH), 7.3 (d, J = 8 Hz, 2 H, Ar), 8.15 (d, J = 8 Hz, 2 H, Ar).

Reduction of Benzophenone to Benzhydrol (Procedure B). 4-(4-Chloro-α-hydroxybenzyl)phenyl β-D-Xylopyranoside (81). To a suspension of 5 g (13.7 mmol) of 57 in methanol (120 mL) was added $NaBH_4$ (0.55 g, 14 mmol) portionwise. The

Table V



no.	position of β-D-Xylp	R'1	R'2	R1	mp, °C	formula	anal.	$[\alpha]^{20}_D$, deg	X	LD_0	activity,[a] %
76	4	2'-Cl	4'-Cl	H	152	$C_{18}H_{18}Cl_2O_5$	CH	-17 (c 0.5, MeOH)	CHOH	350[b]	37
77	4	H	H	H	190	$C_{18}H_{20}O_6$	CH	-26 (c 0.5, MeOH)	CHOH	>800	37
78	4	3'-NO_2	H	H	125	$C_{18}H_{19}NO_6$	CHN	-15 (c 0.5, MeOH)	CHOH	600[b]	63
79	4	4'-NO_2	H	H	142	$C_{18}H_{19}NO_6$	CHN	-17 (c 0.5, MeOH)	CHOH	>800	80
80	4	2'-Cl	H	H	210	$C_{18}H_{19}NO_6$	CH	-23 (c 0.5, MeOH)	CHOH	>800	51
81	4	4'-Cl	H	H	182	$C_{18}H_{19}NO_6$	CHCl	-22 (c 0.5, MeOH)	CHOH	>800	39
82	4	2'-CH_3	H	3-CH_3	110	$C_{20}H_{24}O_6$	CH	-9.5 (c 0.4, MeOH)	CHOH	450[b]	14 (NS)[c]
83	4	4'-OCH_3	H	H	153	$C_{19}H_{22}O_6$	CH	-28 (c 0.5, MeOH)	CH_2	>800	43[c]
84	4	H	H	H	160	$C_{18}H_{20}O_6$	CH	-28 (c 0.5, MeOH)	CH_2	>800	12 (NS)[c]
85	4	3'-NO_2	H	H	139	$C_{18}H_{19}NO_7$	CHN	-24 (c 0.5, MeOH)	CH_2	>800	32 (NS)[c]
86	4	4'-NO_2	H	H	166	$C_{18}H_{19}NO_7$	CHN	-20 (c 0.5, MeOH)	CH_2	650	80[c]
87	4	2'-Cl	H	H	183	$C_{18}H_{19}ClO_5$	CHCl	-27 (c 0.5, MeOH)	CH_2	>800	0[c]
88	4	4'-Cl	H	H	142	$C_{18}H_{19}ClO_5$	CH	-25 (c 0.5, MeOH)	CH_2	>800	22[c]
89	4	2'-Cl	4'-Cl	H	158	$C_{18}H_{18}Cl_2O_5$	CH	-26 (c 0.5, MeOH)	CH_2	640[b]	25 (NS)[c]
90	3	4'-Cl	H	H	84	$C_{18}H_{19}ClO_6 \cdot {}^1/_2H_2O$	CH	-37 (c 0.5, MeOH)	CHOH	>800	0
91	3	H	H	H	168	$C_{18}H_{20}O_6$	CH	-2 (c 0.5, MeOH)	CHOH	>800	19 (NS)[c]
92	3	4'-Cl	H	H	136	$C_{18}H_{19}ClO_5$	CH	-35 (c 0.5, MeOH)	CH_2	500[b]	46[c]
93	3	H	H	H	140	$C_{18}H_{20}O_5$	CH	-38 (c 0.5, MeOH)	CH_2	200[b]	39 (NS)[c]

[a] See Table I. [b] LD_{50}. [c] The injection of the stimulus was 4 h after administration of the tested compound otherwise the injection of the thrombogenic agent was 2 h later. NS = not significant.

Table VI

	79[b]	heparin[c]
ED_{50},[a] mg/kg iv	17.8 (14.5–21.1)	0.15 (0.12–0.17)

[a] Calculated by linear regression with their corresponding 95% confidence limits (in parentheses). [b] 79 was administered by iv route before injection of thrombogenic agent (Wessler Xa). [c] Obtained from Terhormon, Novara, Italy.

mixture was stirred for 1 h at room temperature and the solution then deionized by adding Amberlite IR-120 (H^+) cation-exchange resin until the pH became neutral. The diastereoisomeric mixture thus formed was chromatographed, after evaporation of the solvent, on a column of silica gel (3:1 toluene–MeOH) to yield 4.33 g (87%) of 81 as a mixture of epimers: mp 182 °C; $[\alpha]^{20}_D$ -22° (c 0.5, MeOH); ^1H NMR (Me_2SO-d_6) δ 3.3 (m, 4 H), 3.7 (m, 1 H), 4.8 (d, $J_{1,2}$ = 7.04 Hz, 1 H, H-1), 5.0 (broad, 1 H), 5.3 (broad, 1 H), 5.6 (d, J = 3 Hz, 1 H), 5.9 (d, J = 3 Hz, 1 H), 6.9 (d, J = 9.5 Hz, 2 H, Ar), 7.26 (d, J = 9.5 Hz, 2 H, Ar), 7.35 (s, 4 H, Ar).

Zemplén Deacylation. 4-(4-Chlorobenzoyl)phenyl β-D-Xylopyranoside (57). A solution of 16 (3.4 g, 7 mmol) in dry methanol (50 mL) was stirred with a 3 M solution of methanolic sodium methoxide (0.5 mL) for 2 h at room temperature. The solution was deionized by addition of Amberlite IR-120 (H^+) cation-exchange resin, the resin filtered off, and the filtrate taken to dryness. The residue was recrystallized from methanol to yield 2.3 g (88%) of 57: $[\alpha]^{20}_D$ -27° (c 0.1, MeOH); ^1H NMR (Me_2SO-d_6) δ 3.34 (m, 1 H), 3.8 (m, 1 H), 5.1 (m, 3 H), 5.4 (d, $J_{1,2}$ = 4.8 Hz, 1 H, H-1), 7.17 (d, J = 8 Hz, 2 H, Ar), 7.52–7.84 (m, 6 H, Ar).

α-(3-Methoxyphenyl)-4-morpholinoacetonitrile. To a mixture of 3-methoxybenzaldehyde (3 g, 22 mmol), p-toluenesulfonic acid (4 g, 27 mmol), and morpholine (3.8 g, 44 mmol) was added potassium cyanide (1.43 g, 22 mmol). The mixture was stirred for 3 h under reflux. After neutralization with a concentrated solution of $NaHCO_3$ and extraction with ethyl acetate, the organic layer was washed with water until the pH was neutral. Ethyl acetate was removed by evaporation under diminished pressure, and the solid residue was recrystallized from isopropyl ether to yield 4.12 g (81%), mp 40 °C.

3-Methoxy-4'-nitrobenzophenone. The preceding compound (4.12 g, 18 mmol) was added portionwise to a suspension of sodium hydride (0.53 g, 18 mmol) and N,N-dimethylformamide (40 mL) at 0 °C. After 30 min at this temperature, a solution of 1-fluoro-4-nitrobenzene 2.5 g (18 mmol) in 20 mL of N,N-dimethylformamide was added slowly. The mixture was stirred at room temperature until the starting materials had disappeared. After hydrolysis on ice, the precipitate was removed by filtration and washed with water until the pH was neutral. The crude, wet material was stirred under reflux for 1 h in 40 mL of 70% aqueous acetic acid. From this hydrolyzed product the residue was removed by filtration and washed with water until the pH was neutral to give 3.2 g (70%) of the final compound. This pure compound was used directly for the next step.

4'-Nitro-3-hydroxybenzophenone. The preceding compound (3 g, 11.6 mmol) was stirred under reflux for 4 h in 5 mL of acetic acid and 6 mL of 62% hydrogen bromide. The acid medium was hydrolyzed with cold water, the residue removed by filtration, and the product recrystallized from isopropyl ether to give 2.03 g (70%) of the product, mp 116 °C.

General Method for Synthesis of Benzophenone from Grignard Reagent: Preparation of 2'-Methyl-3-methoxybenzophenone. Under an atmosphere of nitrogen 9.35 g (50 mmol) of 3-bromoanisole dissolved in 5 mL of anhydrous THF was added slowly to a suspension of 1.8 g (75 mmol) of magnesium in 5 mL of THF. The reflux was maintained during the addition of 3-bromoanisole until the magnesium had disappeared. This Grignard reagent was added slowly to a solution of 2-methylbenzoyl chloride (7.22 g, 50 mmol) in 10 mL of THF at -20 °C. The reaction was stirred overnight at room temperature and then hydrolyzed with a cooled solution of M HCl. The aqueous solution was extracted with ethyl ether. The organic layer was washed with water until the pH was neutral, dried over $MgSO_4$, and evaporated under diminished pressure to yield 1.22 g (86%) of this crude compound that was used directly for the next step.

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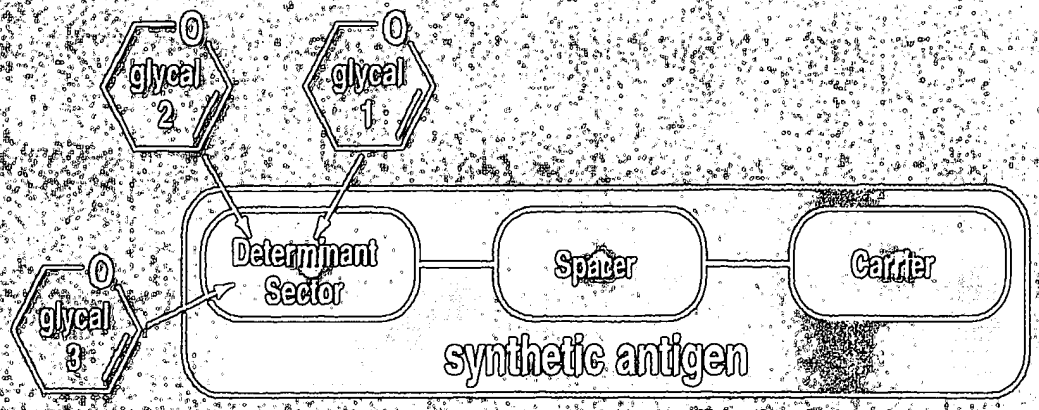
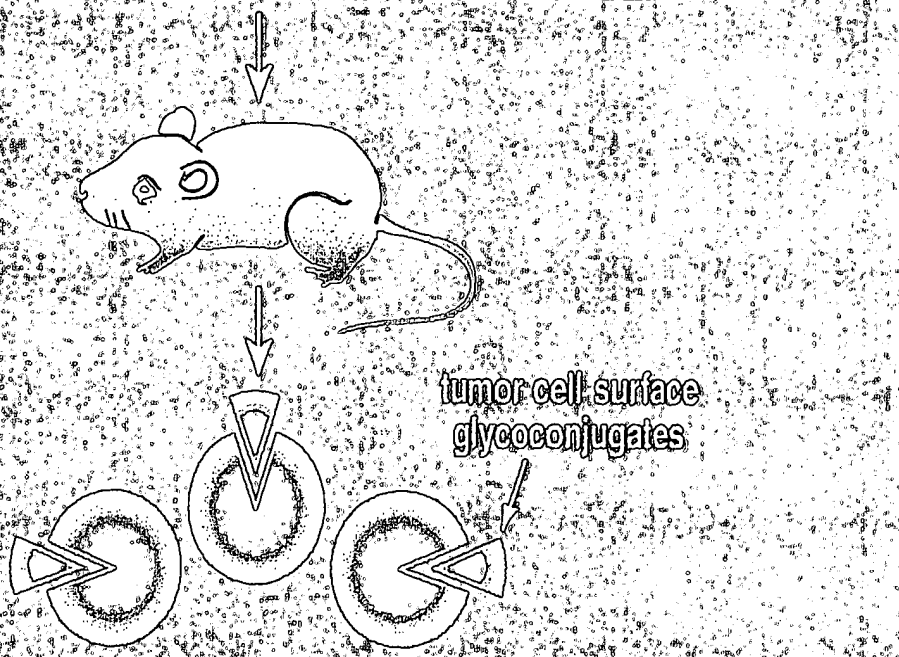
Glycal Assembly





mouse antibodies bind to tumor transformed cell lines

Glycals in Organic Synthesis: The Evolution of Comprehensive Strategies for the Assembly of Oligosaccharides and Glycoconjugates of Biological Consequence

Samuel J. Danishefsky and Mark T. Bilodeau

This review provides a personal account of the explorations of a research group in oligosaccharide and glycoconjugate construction. The journey began twenty years ago with the study of Diels–Alder reactions of complex dienes. By extending this methodology to aldehydo-type heterodienophile equivalents, access to unnatural glycals was gained (LACDAC reaction). From this point a broad-ranging investigation of the use of glycals in the synthesis of oligosaccharides and other glycoconjugates was begun. Mobilization of glycals both as glycosyl donors and glycosyl acceptors led to the strategy of glycal assembly. Several new glycosylation techniques were developed to provide practical underpinning for this logic of glycal assembly. Glycal-based paradigms have been shown to be nicely adaptable to solid phase supported synthesis. Moreover, glycal assembly—both in solution and on solid phases—has been used to gain relatively concise and efficient entry to a variety of biologically interesting and potentially valuable constructs. Some of these syntheses, particularly in the field of tumor antigens, have led to novel compounds which are in the final stages of preclinical assessment. This review presents an account of the chemical reasoning at the center of the program.

Keywords: glycals · glycoconjugates · glycosylations · oligosaccharides · synthetic methods

1. Background and Settings

The synthesis of carbohydrate-based structures is emerging as a major frontier area for organic chemistry. In addition to their well-appreciated roles in supporting structural matrices, in energy storage, and as biosynthetic starting materials, carbohydrates are cast in a variety of interesting settings as glycoconjugates, for example as antibiotics,[1] antitumor agents,[2] and cardiotonic glycosides.[3] The gangliosides are being increasingly implicated as tumor antigens and cellular differentiation markers.[4] The importance of the carbohydrate domains in glycoproteins and glycolipids as elements in cell surface recognition is manifested by their role in cellular adhesion[5, 6] and as determinants in blood group typing.[7] Another incentive for focusing on carbohydrates is their usefulness as enantiomerically pure starting materials for the synthesis of various natural products and other types of target molecules.[8]

Our first encounter with carbohydrate chemistry arose from happenstance rather than through careful planning. A slight digression may be appropriate. From 1974 to 1981 we had been investigating the synthetic potential of the uncatalyzed Diels–Alder reaction of siloxydienes with electrophilic dienophiles.[9, 10] Actually the problem that triggered those studies was a proposed synthesis of vernolepin.[11] For that purpose we thought that intermediate **4** might be useful (Scheme 1). It seemed to have all of the functionality necessary to allow for a plausible route to our target (the dotted arrows highlight transformations that were eventually achieved). At the time, however, there was no known route to efficiently introduce the $\Delta^{1,2}$ conjugated double bond via a saturated 3-ketone in the *cis*-fused series of compounds.

This problem prompted the idea of using the parent version of diene **1** (R^1, $R^2 = H$) in a Diels–Alder reaction with dienophile **3** (itself the product of the Diels–Alder reaction of 1,3-butadiene with methyl propiolate). The synergism of the 1,3-substituents on **1** (R^1, $R^2 = H$) facilitated reaction with the sluggish dienophile **3**. Furthermore the adduct could be easily transformed into the desired **4**.

The matching of electronically complementary Diels–Alder components did indeed favor many otherwise difficult cycloadditions. By taking advantage of functional groups in the diene component at the points of linkage (for example in **3**, Scheme 1), we were able to realize the total synthesis of a variety of natural products, including vernolepin. Some of the other targets were also quite challenging, and their total syntheses were not without instructional value (cf. prephenic acid (Scheme 1), pentalenolactone, and griseofulvin).

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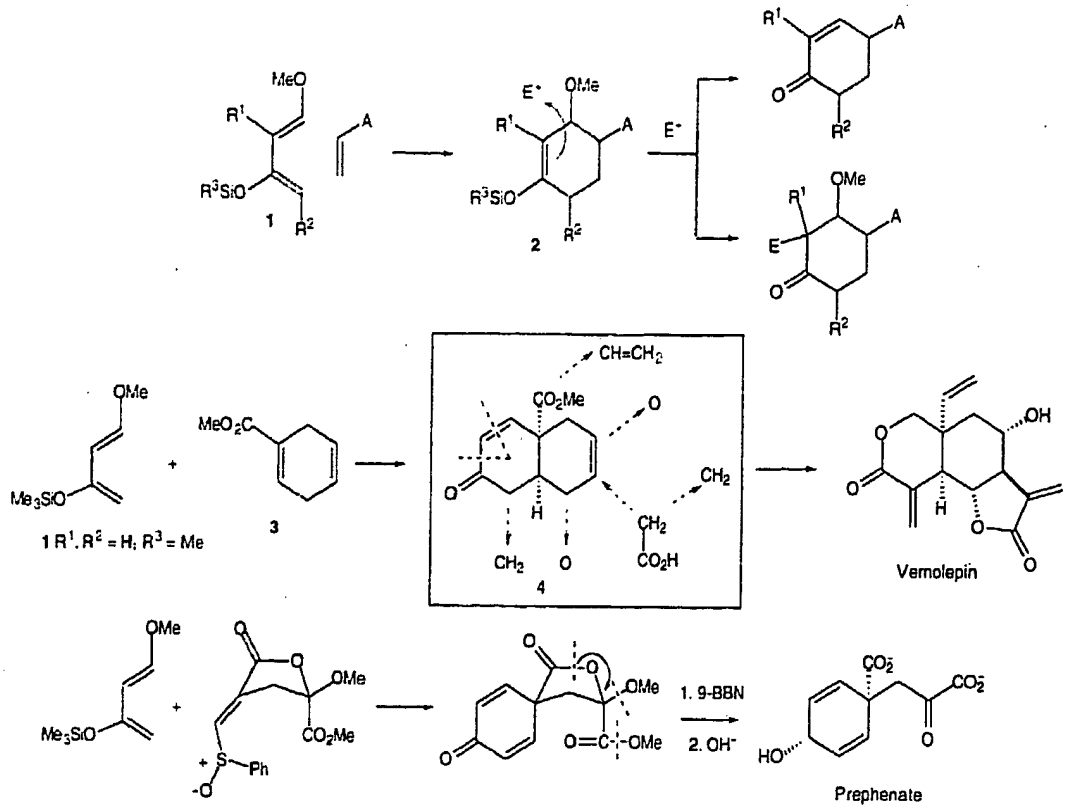

Scheme 1. Diels–Alder reactions of siloxydienes with electrophilic dienophiles (A = electron-withdrawing substituent. E^+ = electrophile, including H^+).

Perhaps the most enduring aspect of this early work in Pittsburgh was the interest it spawned in diene 1. in the broad class of siloxydienes and, more generally, in the concept of heavily functionalized dienes in the Diels–Alder reaction. While these ideas are now taken for granted, much follow-through work was needed for them to gain wide acceptance and usage.[12]

As the use of functionalized dienes in all-carbon Diels–Alder reactions was developing in many other laboratories,[13] we cast our attentions toward a new departure. We came to wonder about the possibility of the cycloaddition of siloxydienes with aldehydes. Elsewhere we have related the early history of this reaction.[14] While it was known that aldehydes especially activated by adjacent electron-withdrawing groups (e.g. α-dicarbonyl systems or α-polyhaloaldehydes) would undergo cycloaddition with "nucleophilic" dienes,[15] analogous processes were not realizable with typical aldehydes under thermal conditions. It remained for James Kerwin, then a graduate student in our laboratory at Yale University, to discover that under appropri-

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M. T. Bilodeau

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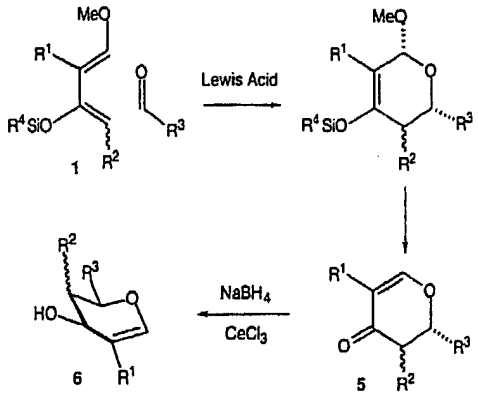
ate Lewis acid catalysis, cyclocondensation between suitable siloxydienes and aldehydes was in fact a general and reliable reaction.[16] After several years of investigation the cyclocondensation reaction emerged as a central element in a new strategy for the synthesis of polyoxygenated natural products.[17]

2. The Synthesis of Enantiomerically Enriched Artificial Glycals and Applications to Total Synthesis

The Lewis acid catalyzed diene–aldehyde cyclocondensation (LACDAC) reaction provided a rapid route to novel dihydropyrones of the type **5** (Scheme 2). It was subsequently shown, first



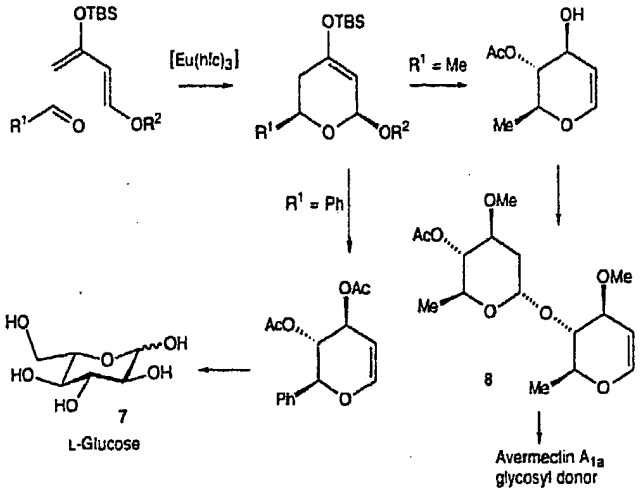
Scheme 2. Synthesis of glycals by the LACDAC reaction (β-R^2: gluco series; α-R^2: galacto series).

by Toby Sommer, that 1,2-reduction of such dihydropyrones with sodium borohydride mediated by cerium(III) chloride gave alcohols such as **6** in which the hydroxyl group is in equatorial position (Scheme 2).[18] We began to refer to such compounds as "glycals." The term had been reserved by carbohydrate chemists for naturally derived pyranose or furanose systems bearing a C1–C2 double bond. The possibility that racemic compounds such as **6**, derived by purely synthetic means, might be described as "glycals" was greeted not surprisingly with reservation by some in the community of traditional carbohydrate chemists, but to us, the connection seemed obvious.

Four pathways have subsequently been followed to produce enantiomerically enriched or enantiomerically pure dihydropyrones and glycals by means of the LACDAC reaction.[14] We review them here chronologically.

Early, but only modest success was achieved by using enantiomerically pure oxophilic lanthanide complexes as Lewis acid catalysts in the cyclocondensation reaction.[19] Lanthanides bearing chiral ligands had, of course, previously been used as chemical shift reagents. It is often overlooked but perhaps worth remembering that the experiments of Mark Bednarski, wherein [Eu(hfc)$_3$] imparted enantioselectivity in the LACDAC reaction, were among the first examples of decent enantiotopic induction in the metal-catalyzed formation of carbon–carbon bonds (hfc = 3-(heptafluoropropyl(hydroxymethylene)-D-camphorato).

In the next phase we investigated the effects of mounting an enantiomerically pure auxiliary on the diene component. Here, only painfully modest seletivities were realized. However, dramatic diastereomeric excesses were obtained by combining the use of a chiral auxiliary with an enantiomerically pure lanthanide complex.[20] After separation of the diastereomeric products, enantiomerically pure versions of dihydropyrones were available. This strategy was used in the synthesis of L-glucose (**7**)[21] and in the construction of the carbohydrate fragment of avermectin A_{1a} (**8**, Scheme 3).[22] An interesting feature here is that the effects of the catalyst and auxiliary are nonmultiplicative. Indeed, Bednarski's best results were obtained when the enantioselective tendencies of the auxiliary and catalyst components of the "*ee* enrichment package" veered in opposite directions.

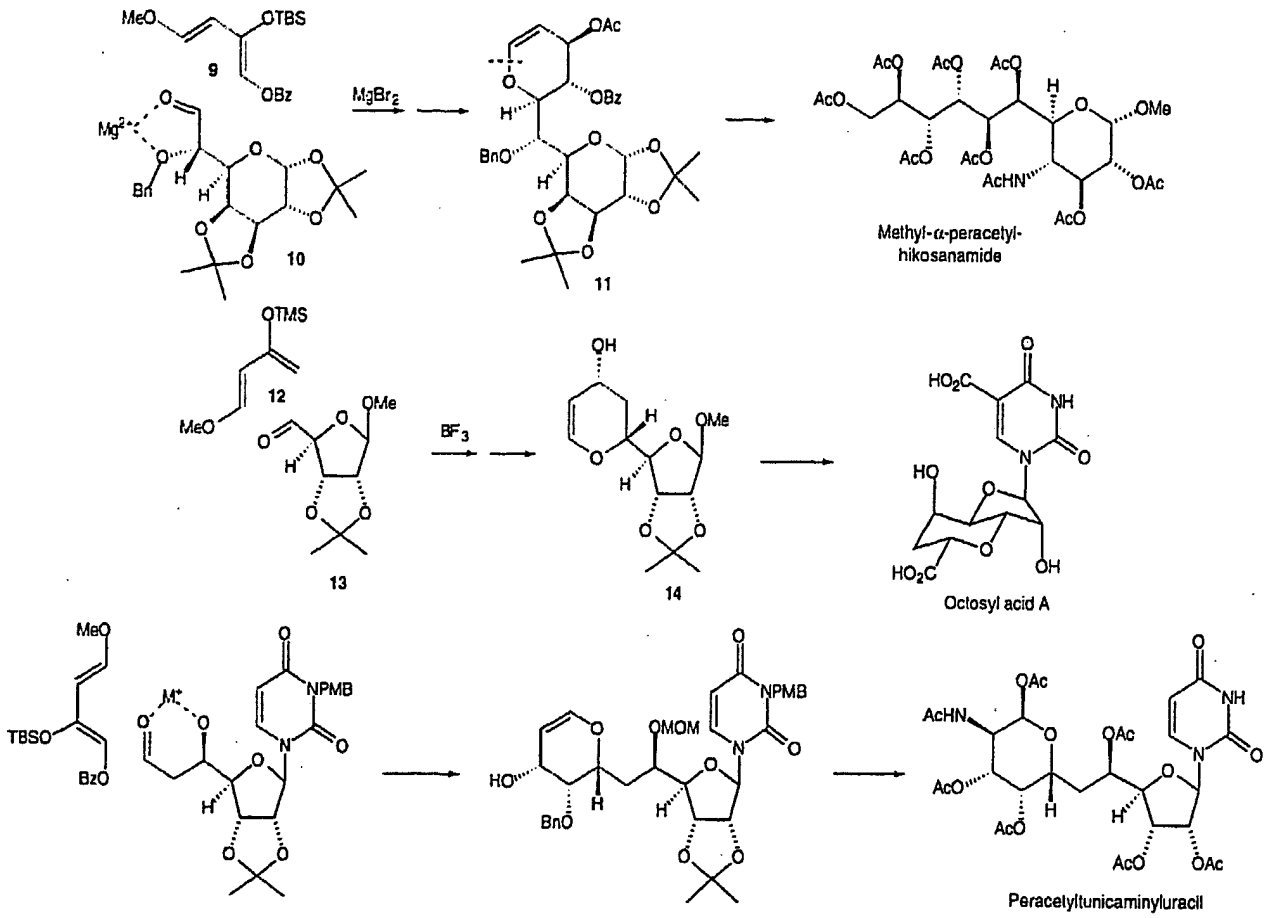


Scheme 3. Enantioselective LACDAC reactions (R^2 = L-8-phenylmenthyl).

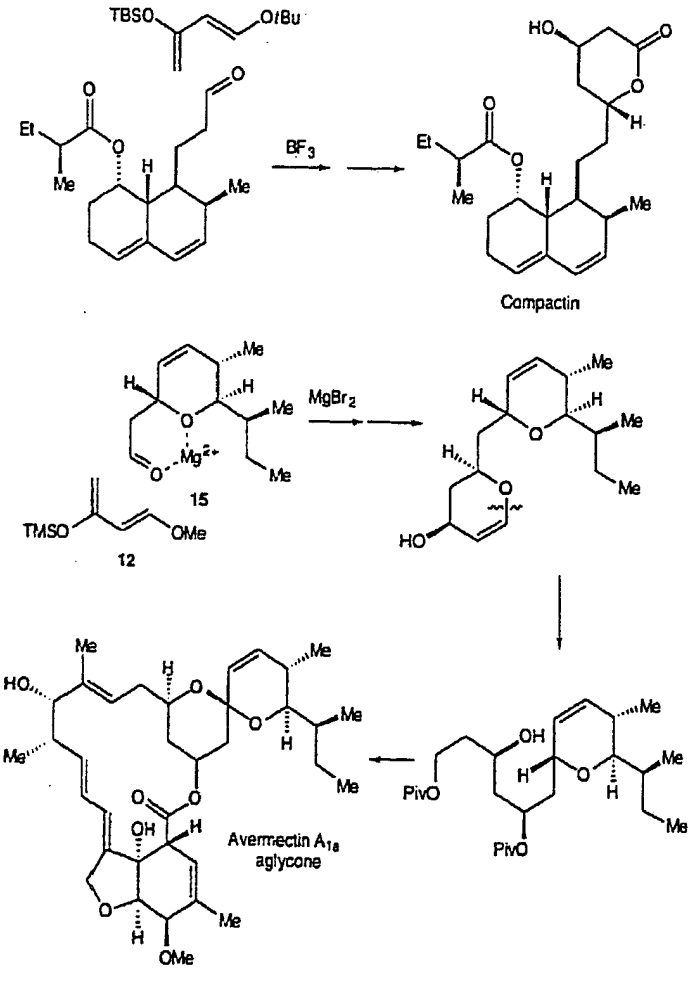
It is well to note that H. Yamamoto and co-workers have pioneered the use of enantiomerically pure BINAP-based Lewis acid catalysts (BINAP = 1,1'-binaphthalene-2,2'-diylbis(diphenylphosphane), which confer high enantioselectivity on the LACDAC reaction.[14, 23] This chemistry, in conjunction with the stereoselective reduction of dihydropyrones by the Luche system ($NaBH_4/CeCl_3$), constitutes an excellent route for the formation of novel enantiomerically pure artificial glycals.

Unlike these methods, in which the de novo induction of chirality in the dihydropyrone was attempted, the third approach started with enantiomerically pure aldehydes. The proximal stereogenic center in these aldehyde heterodienophiles imparted face selectivity to the LACDAC reaction. In this way various complex optically pure glycals were obtained from precursors that were formed in high diastereomeric excesses. This chemistry allowed us to achieve synthetic routes to the higher order monosaccharides[24] including lincosamine,[25] *N*-acetylneuraminic acid,[26] *rac*-3-deoxy-*manno*-2-octulosonic acid (KDO),[26] hikosamine,[27] and octosyl acid A.[28] In some cases (**11** → α-methylhikosanamide and **14** → octosyl acid, Scheme 4) the pyrone substructure served as a temporary locus for establishing configurational relationships which were unveiled upon opening of the ring.

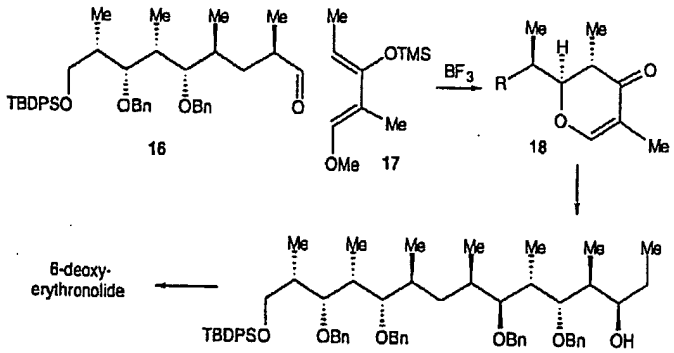
Most of our advances in systems bearing resident chirality were achieved with aldehydes in which the stereogenic centers contain electron-withdrawing groups α to the formyl function (cf. **9** + **10** → **11**; **12** + **13** → **14**, Scheme 4). However, some striking successes were realized even when the resident chirality was more remote from the site of induction. In our syntheses of tunicaminyluracil (Scheme 4)[29] and compactin (Scheme 5)[30]



Scheme 4. Applications of the LACDAC reaction to total synthesis.



significant diastereoselectivities were attained in the absence of an α-directing function. Another important case of strong selectivity, involving resident chirality at some distance from the site of bond formation, arose during the LACDAC reaction of **12** and **15**. This was an important step en route to our total synthesis of the aglycone of avermectin A_{1a} (Scheme 5).[22] Another particularly important demonstration was realized during our synthesis of 6-deoxyerythronolide B (**16** + **17** → **18**, Scheme 6),

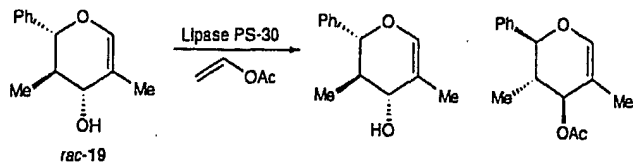


Scheme 6. Application of the LACDAC reaction to the synthesis of 6-deoxyerythronolide.

wherein the stereogenicity seems to be enhanced by stereocenters remote from the aldehyde.[31] It appears probable that new potentials for face selectivity in the LACDAC reaction still remain to be identified and tapped.

As previously mentioned, Sommer had demonstrated that reduction of dihydropyrones with the Luche system ($NaBH_4$/

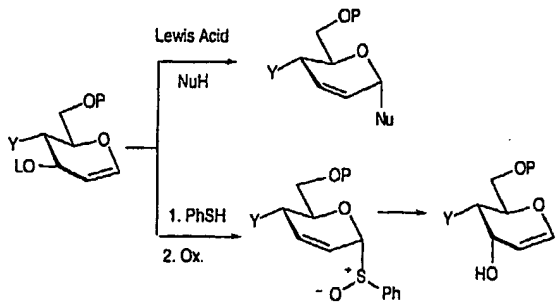
group at C3 and is not significantly complicated by 1,4-addition.[18] This chemistry paved the way for the fourth and most general route to enantiomerically pure artificial glycals.[32, 33] Thus, David Berkowitz found that racemic compounds of the type 19 could be kinetically resolved by enantioselective acetylation exploiting the Wong method (lipase PS-30 and vinyl acetate as the acylating agent, Scheme 7).[34]



Scheme 7. Enzymatic resolution of a racemic glycal.

Our enthusiasm for the LACDAC methodology notwithstanding, naturally occurring sugars are still the best source of glycals bearing the usual hexose functionality at C3, C4, and C6. In particular, glycals closely related to D-glucal, D-galactal, and D- or L-fucal are readily accessible from commercially available precursors. It is only when the required functionality of the target glycal is not reasonably accessible from carbohydrates that total synthesis by means of LACDAC chemistry can be superior to partial synthesis.

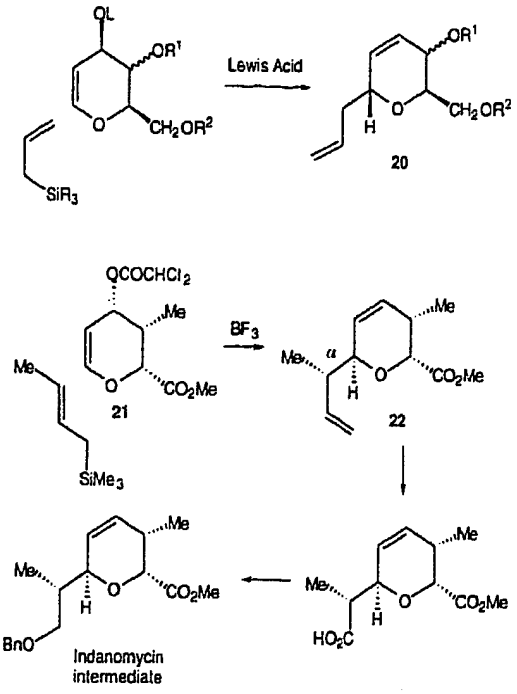
The synthesis of glycals from carbohydrate precursors bearing a axial hydroxyl group at C3 (for example D-allal and D-gulal) can be achieved by reductive elimination of hetero groups at C1 and C2.[35] However, in several important instances the starting hexoses bearing axial hydroxyl groups at C3 were themselves accessible only with extreme difficulty. A route was devised to deal with this type of situation. Our method exploits a form of the Ferrier-type displacement of glycals.[36, 37] As will be seen, the classical Ferrier rearrangement leading to pseudoglycals was valuable to our developing program (Scheme 8).



Scheme 8. Ferrier rearrangements of glycals and utilization of subsequent sulfoxide rearrangements (LO = leaving group, Y = α-OR for allals, Y = β-OR for gulals).

For the case at hand, reaction of a C3 equatorial glycal with thiophenol gives rise to an axial thiophenyl "pseudoglycal," which is converted by oxidation into a C3 axial glycal, presumably by rearrangement of its sulfoxide.[38, 39] While the scope, limitations, and mechanism of this reaction have not been fully defined, it has already found application in our total synthesis of the trisaccharide sector of esperamicin and the aryltetrasaccharide sector of calicheamicin (see Schemes 59 and 60, respectively).

As access to glycals was being significantly improved, and as our syntheses of many of the higher order monosaccharides were being concluded, we began to explore new options for using glycals as synthetic building blocks in the construction of various glyconjugates. Before turning to the main theme of this report, we will review the application of glycals to the stereoselective synthesis of C-glycosides. A key reaction developed can be regarded as a Lewis acid promoted "carbon-Ferrier" process.[40] Thus reactions of allyltrimethylsilane with activated glucal or galactal derivatives afforded C-glycosides bearing axial allyl functions (20, Scheme 9).

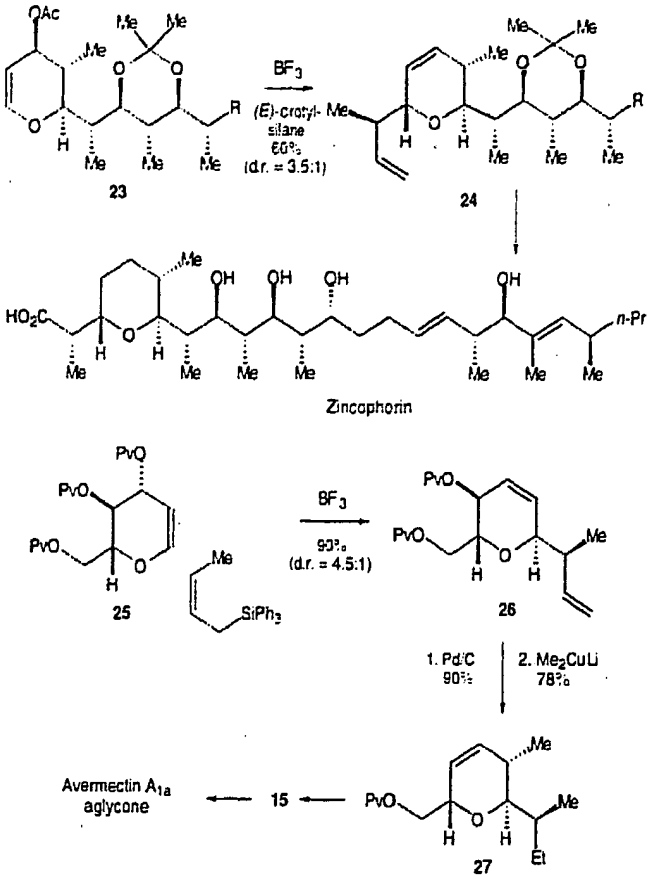


Scheme 9. Allylsilanes employed in the Ferrier rearrangement (OL = leaving group, α-OR^1: gluco series, β-OR^1: galacto series).

Subsequently, it was found that reaction of various glycals with *cis*- and *trans*-crotylsilanes affords methallylated glycosides with good stereoselectivitiy at C_2. The resulting configuration is a function of the geometry of the crotyl group. This capability was used to good effect in our syntheses of indanomycin,[41] zincophorin,[42] and avermectin A_{1a}.[22] Thus, reaction of *trans*-crotylsilane with glycals 21 (Scheme 9) and 23 (Scheme 10) gave rise to 22 and 24 as the major products, respectively. However, triphenyl(*cis*-crotyl)silane reacted with glycal 25 to give rise to 26 (Scheme 10). The latter was reduced then treated with lithium dimethylcuprate to provide 27, from which we were able to reach the avermectin aglycone via 15.

3. Glycals in the Synthesis of Oligosaccharides—Some Early Notions

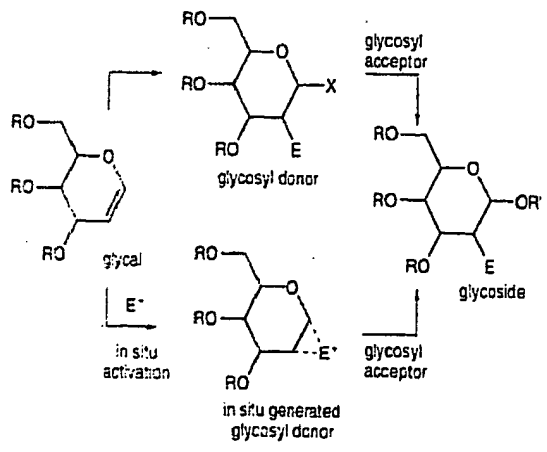
We now turn to the use of glycals in the general construction of oligosaccharides and other glyconjugates. To facilitate discourse, we make reference to a useful terminological distinction that has evolved to describe the two components entering into a glycosylation reaction. Thus, the component that contributes



Scheme 10. The use of the allylsilane Ferrier rearrangement in total synthesis.

the anomeric carbon of the resultant glycoside is described as the glycosyl donor (Scheme 11). The donor reacts with a glycosyl acceptor to establish a glycoside. In the overwhelming majority of glycosylation reactions, the acceptor is a nucleophile that furnishes the oxygen of the resultant glycoside by replacement of a leaving group at the anomeric carbon of the electrophilic glycosyl donor. However, the novel glycosylations of Schmidt,[43] David and Lubineau,[44] Vasella,[45] and Kahne[46] attest to the need to decouple the terms "glycosyl donor" and "glycosyl acceptor" from mechanistic descriptors such as "nucleophile" and "electrophile".

It is also well to distinguish two modalities by which glycals can function as glycosyl donors (Scheme 11). In one motif the glycal is first converted, through a reaction or sequence of reactions, into an isolable or at least identifiable glycosyl donor (for



instance, by epoxidation, azidonitration,[47] or sulfonamidoglycosylation, vide infra). In essence the glycal is a precursor to a structurally defined glycosyl donor. Alternatively, in situ electrophilic activation might mobilize the glycal to function as the donor in the form of a substoichiometric nonisolable intermediate rather than as a defined reaction component.

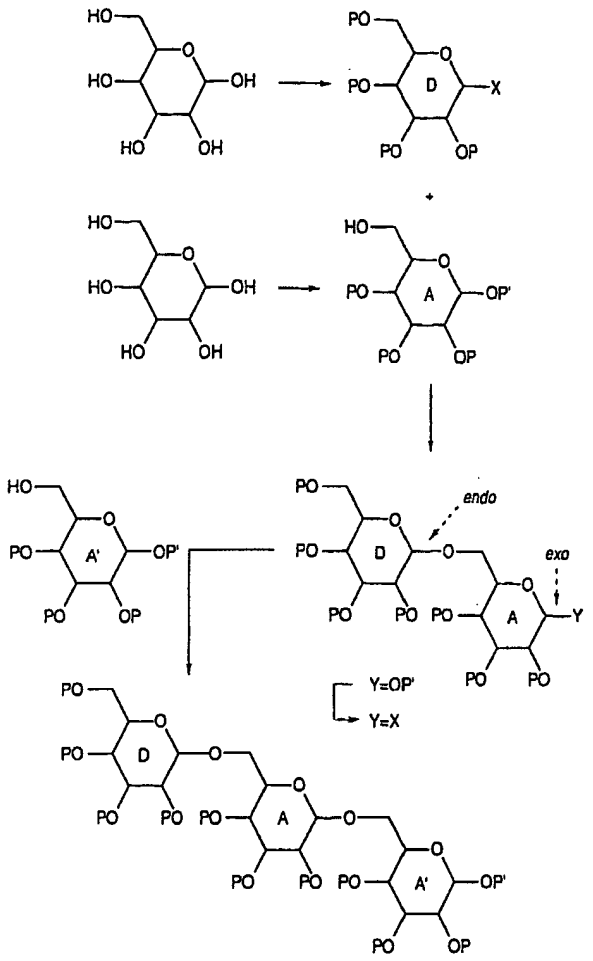
The possibility of utilizing glycals as glycosyl donors in disaccharide synthesis had been demonstrated in the pioneering research of Lemieux[48] and Thiem[49] by halonium-mediated coupling to suitably disposed acceptors. These particular reactions had been shown by Thiem[49] to have a high proclivity for *trans*-diaxial addition and provided a crucial route to α-linked disaccharides bearing an axial 2-iodo function on the nonreducing end. Owing to the difficulty in effecting nucleophilic displacement of the iodine in such systems,[50] the Thiem chemistry has thus far found its most useful application in the synthesis of 2-deoxyglycosides.[49, 51]

Our chemistry, directed toward assembly of glycoconjugates and oligosaccharides, came to be organized around four questions: 1) Could a glycal linkage at the terminating end of a di- or oligosaccharide serve as a useful glycosyl donor (by either of the modalities discussed above)? 2) Could glycals also function as glycosyl acceptors? Surprisingly, prior to our investigation this question had not been examined. 3) Could unnatural glycals available through LACDAC chemistry serve as stereoselective glycosyl donors and acceptors? 4) Would the glycal-based methodology be of sufficient scope to find useful application in the total synthesis of complex multifunctional targets? The translatability of advances from the relatively sheltered world of "synthetic demonstrations" to the often harsh and more demanding realities of the total synthesis of complex targets cannot be assumed as a matter of course!

If glycals could serve both as glycosyl donors and as glycosyl acceptors in a broad range of couplings, a reiterative strategy for the syntheses of complex glycoconjugates, including oligosaccharides, could be contemplated. A potentially important advantage of glycal-based glycosylations was to be the simplification of achieving differentiated hydroxyl protection and presentation. This is readily appreciated by comparison of Schemes 12 and 13.

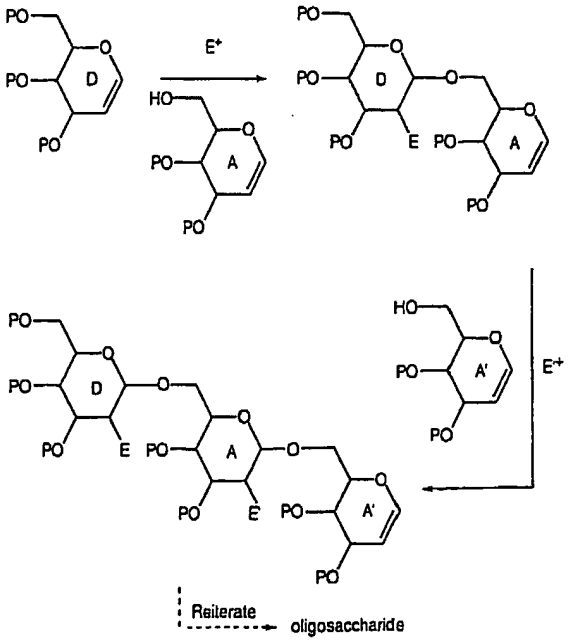
Scheme 12 portrays the classical strategy of glycosylation[52] using fully oxygenated pyranose donors and acceptors. In the very simple case of coupling hexoses D (donor) and A (acceptor) to produce the protected DA disaccharide, several challenges must be overcome. The anomeric hydroxyl function in the eventual donor sugar must be distinguished as a leaving group from the other four hydroxyls. In the eventual acceptor, a particular free hydroxyl (one of five such groups) must be identified for glycosylation, while the anomeric area of the "acceptor" system is properly protected. If one is to proceed toward the 'DAA' trisaccharide, the "*exo*" glycoside moiety of the DA disaccharide must be distinguished from its "*endo*" counterpart. With this accomplished, a leaving group (a glycosyl-donating function) is installed on the erstwhile A sugar, and this ensemble must be appended to glycosyl acceptor A', in which one of five hydroxyls has been identified as the acceptor for the glycosylation.

We contrast this situation with the projected formation of



Scheme 12. Synthesis of a trisaccharide (DAA') by conventional methods. (The stereochemistry of glycosidic linkages is not implied.) A, A' = acceptor unit, D = donor unit, P = protecting group, X = leaving group.

(Scheme 13). The activatable olefinic linkage of uniformly protected glycal D functions as the donor. One of three hydroxyls of acceptor glycal A is to be presented for glycosylation to give the DA disaccharide. After coupling, the glycal linkage of DA is



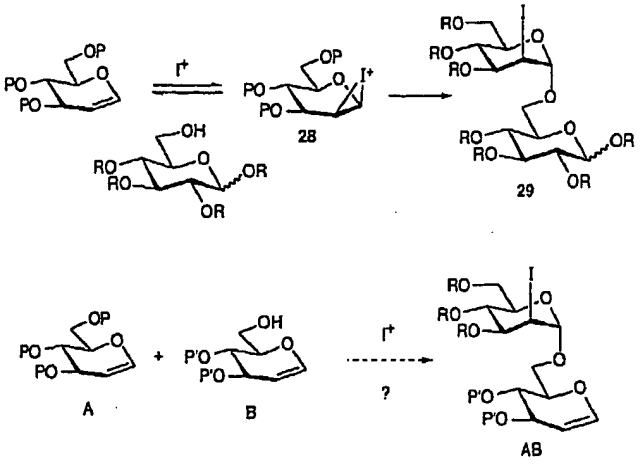
Scheme 13. Synthesis of a trisaccharide (DAA') by a glycal assembly strategy. (The stereochemistry of glycosidic linkages is not implied.) Reiteration of the glycosylation sequence leads to oligosaccharides.

activated (either in situ or in a discrete process) to produce a DA donor vis à vis a new acceptor glycal, A'. In this way, trisaccharide DAA' is obtained. It can be readied for elongation by priming the glycal in the A' sector en route to tetrasaccharide or higher oligomers.

For the reiterative method described in Scheme 13 to be viable and widely applicable, glycals must also function as glycosyl acceptors. Furthermore, for the sorts of extended applications we had begun to contemplate, it would be necessary for glycal linkages at the putative reducing end of larger oligosaccharides to also function as viable donors. For maximum applicability, it would be necessary to fashion a menu of coupling methods in which glycals serve both as donors and as acceptors. To use these concepts in the construction of unnatural glyconjugates and oligosaccharides, unnatural glycals, obtained by synthesis, must be amenable to the methodology being developed.

4. Haloglycosylation

The iodoglycosylation reaction developed by Lemieux[48] and Thiem[49] seemed to represent a particularly difficult challenge to the concurrent use of glycals as acceptors and donors. Classically, iodoglycosylation is carried out with a glycal serving as a donor. The glycal linkage is attacked by an "I^+ equivalent" reagent, for example *N*-iodosuccinimide or *sym*-collidine iodonium perchlorate. In the ordinary case, the presumed substoichiometric intermediate **28** (Scheme 14), arising from the attack of I^+ on the glycal, is attacked by coexisting non-glycal acceptor, in which the reducing end is suitably capped. The stereochemistry of glycosylation is governed by *trans*-diaxial addition and the α-linked disaccharide **29** is produced.

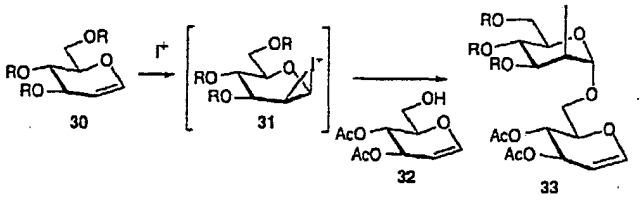


Scheme 14. Iodonium salt mediated coupling of glycals.

We first considered the coupling of two glycals through iodoglycosylation. Such a coupling had apparently not been previously attempted. The problem is controlling the roles of the glycals. Since species **28** is generated as a fleeting intermediate, it seemed very unlikely that the sequential introduction of the two nonidentical glycals (A and B, Scheme 14) and the iodinating agent would control the outcome. Indeed the order of addition of glycals does not determine which one serves as the donor and which as the acceptor en route to the desired AB glycal.

In the case of nonidentical glycals, a particular glycal can be prevented from functioning as a glycosyl acceptor by blocking all of its hydroxyl functions. However, owing to the presence of glycal functionality in the putative acceptor, it can serve as glycosyl donor (see Scheme 14). Therefore, even if one of the two glycals were ineligible as a glycosyl acceptor, significant problems (symmetrical coupling and polymerization) could be encountered in regulating the glycosyl acceptor.

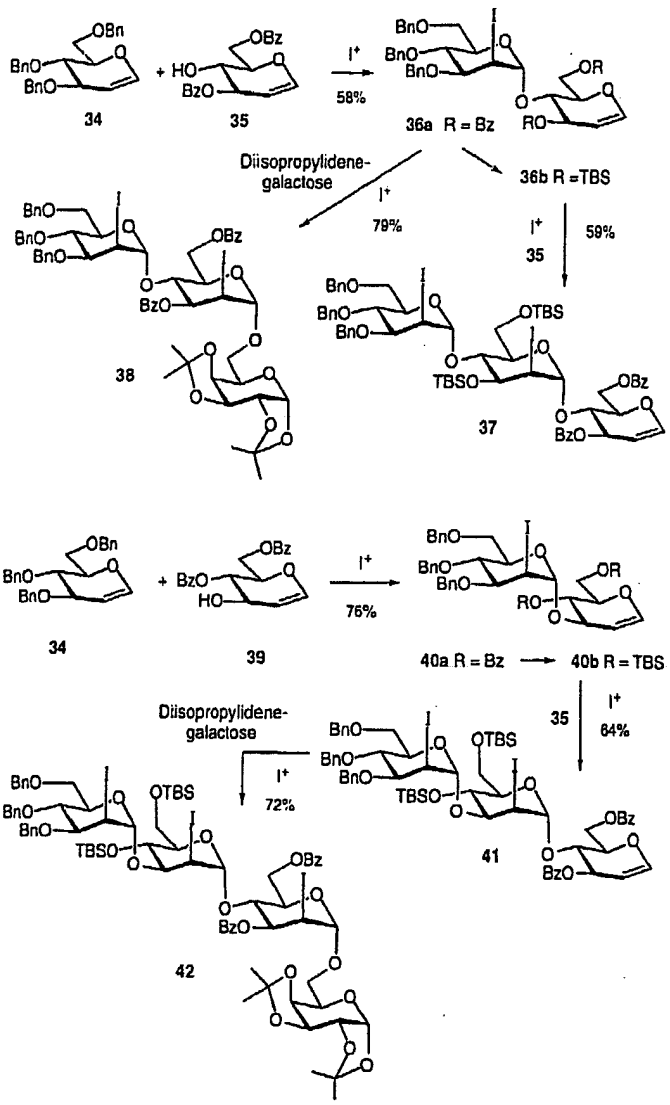
An interesting possible solution presented itself. The thought was that the pattern of protecting groups of the glycal might be used to direct the reaction. While it had long been known that acyl protecting groups lower the reactivity of glycosyl donors,[53a] the idea of exploiting this effect in the coupling of two glycosyl donors had not been conceptualized until the seminal experiments of Fraser-Reid et al.[53b, 54] For the case at hand, consider the two glycals, projected donor 30 and projected acceptor 32 (Scheme 15). The oxygen atoms of 32 are acylated, while those of 30 are alkylated (or silylated). It seemed likely that 30 would be more nucleophilic than 32 towards the iodonium electrophile. Hence, I^+ will attack 30, thereby generating the mechanistically operative glycosyl donor 31. Furthermore, as 30 does not have a free hydroxyl group, it cannot act as an acceptor. Glycal 32, which is equipped with a hydroxyl group, can function as the glycosyl acceptor, giving rise to iodoglycoside 33. For reduction of this concept to practice, it is necessary that the rate of formation and the effective concentration of 31 be much greater than that of the corresponding species derived from 32 or from the disaccharide product 33.



Scheme 15. The use of glycal donors and acceptors in iodoglycosylations (R = alkyl).

The realization of this possibility was achieved by Richard Friesen (34 + 35 → 36, Scheme 16).[55] To reiterate the strategy, it was necessary to enhance the nucleophilicity of the disaccharide glycal toward I^+ so that it would function as a glycosyl donor to the next acceptor, hydroxy diester 35. For this purpose, 36a was converted to 36b. Indeed, iodinative coupling of 36b with 35 gave rise to 37. The analogous coupling of glycal 36a with the capped (non-glycal) acceptor diisopropylidenegalactose was of interest. This case, which led smoothly to 38, again demonstrated (as was already well known from earlier studies) that glycals bearing acyl protecting groups are certainly competent donors in iodoglycosylation reactions with non-glycal acceptors. The essence of Friesen's finding was that the otherwise active 1,2-double bond of the diester-protected glycal substrate does not compete with the analogous double bond in the triether for attack by the iodonium species.

We have not yet elucidated in detail the quantitative effects of the glycal substituents on the iodonium-mediated coupling. The most obvious position where the electronic difference between

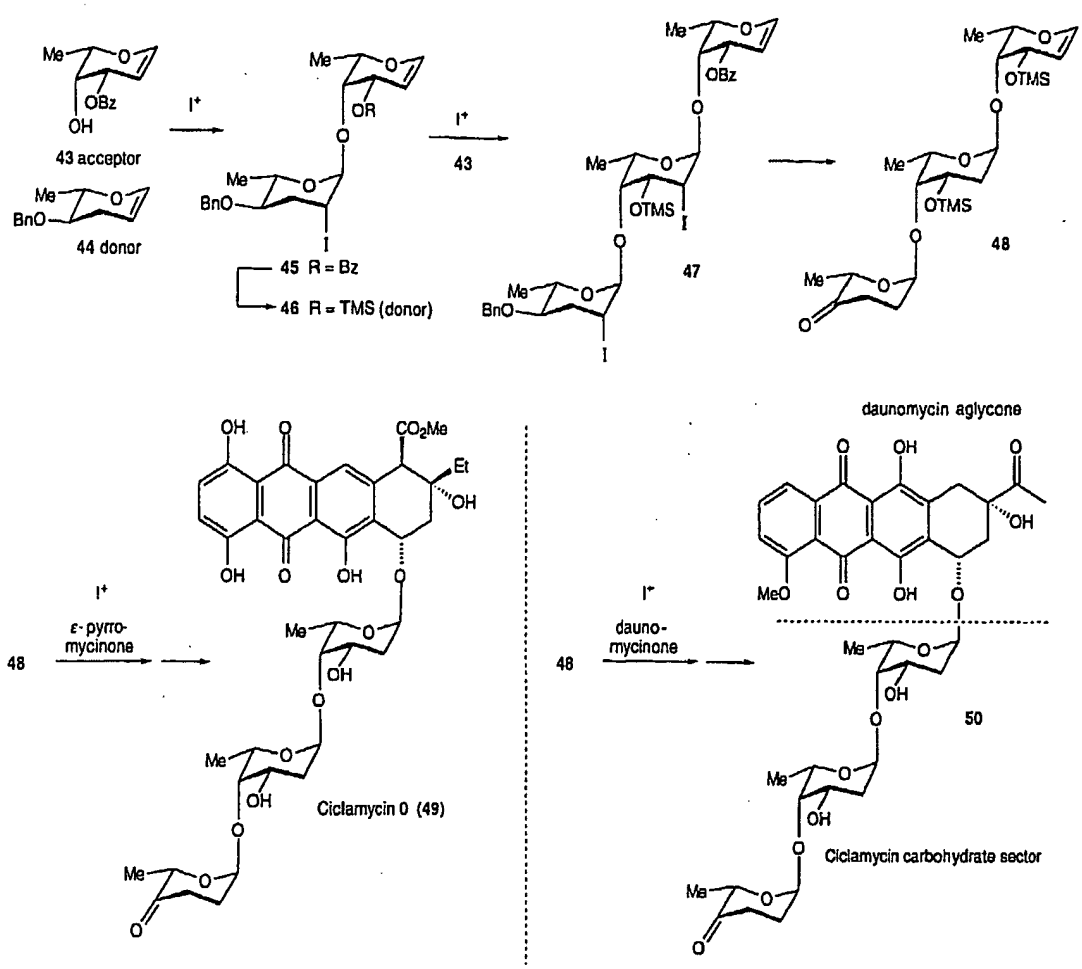


Scheme 16. Polysaccharide assembly employing the iodoglycosylation reaction.

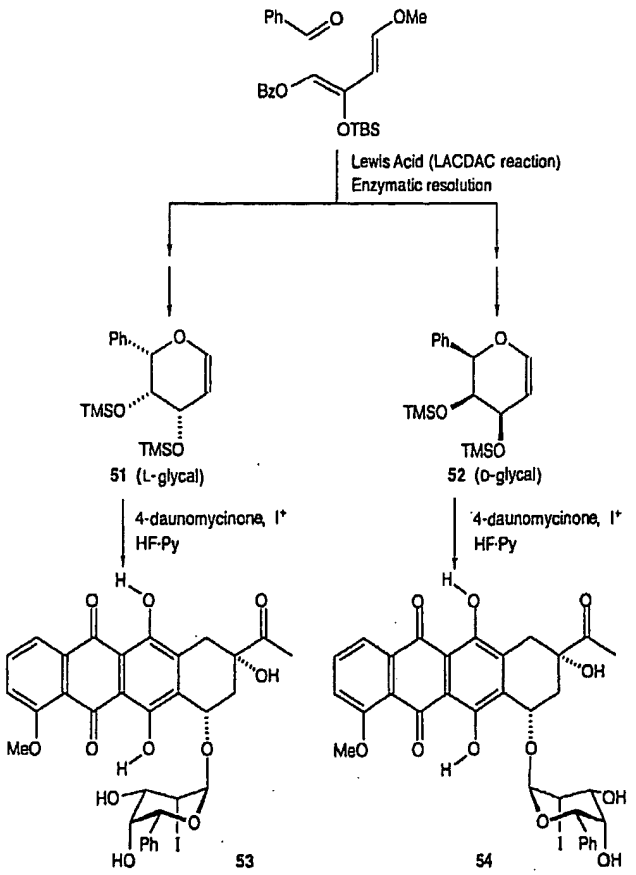
an acyloxy and an alkoxy group would be likely to be decisive would seem to be at the C3 center allylic to the double bond. However, the clean and successful coupling of 34 and 39 to give 40a (Scheme 16) indicates that even without an acyloxy group at C3, subtle effects can be exploited to bring about an orderly progression leading to glycosylation. The disaccharide thus obtained was subjected to the aforementioned procedure (40a → 40b → 41) to provide trisaccharide glycal 41. The trisaccharide 41 was also extended by reaction with diisopropylidenegalactose to give rise to the tetrasaccharide 42.

We were then in a position to exploit this control of glycal–glycal coupling in a synthesis of ciclamycin 0 (Scheme 17).[56, 57] The key coupling steps to prepare trisaccharide glycal 48 are shown. Fortunately, it was feasible to use 48 to iodoglycosylate the ciclamycin 0 aglycone (ε-pyrromycinone), leading after a few steps, to the natural product itself (49). Also, iodonium-mediated coupling of 48 and daunomycinone afforded, after deiodination, the hybrid anthracyclinone 50.

The synthesis of novel anthracyclines using unnatural glycals 51 and 52 (by the LACDAC reaction) has been developed further (Scheme 18).[33] The handedness of the sugar domain (e.g. in 53 and 54) has a profound effect on the DNA binding properties of the modified daunomycinone. This experiment provid



Scheme 17. Synthesis of ciclamicin 0.



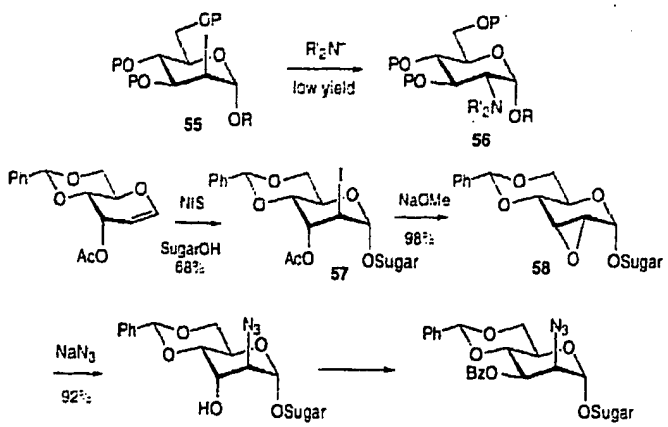
Scheme 18. Enzymatically resolved glycals attached to the daunomycin aglycone. The carbohydrate domain of 53 exhibits the "natural" configuration and is strongly bound to DNA, that of 54 has the opposite configuration and is weakly bound to DNA. Py = pyridine.

ed us with the first sense of the potentially important role of the carbohydrate sector in mediating interactions in the DNA–drug effector region. This critical lesson was not forgotten when we worked in the calicheamicin series (see Section 9).

By combining access to unnatural glycals in optically pure form (in either enantiomeric series), with the capacity for iodoglycosylation, it will be possible to explore in detail the role of the carbohydrate sector in DNA recognition and in the cytotoxicity of the anthracycline antitumor agents. Work in this general direction is now in progress in our laboratory.

5. Azaglycosylation of Glycals

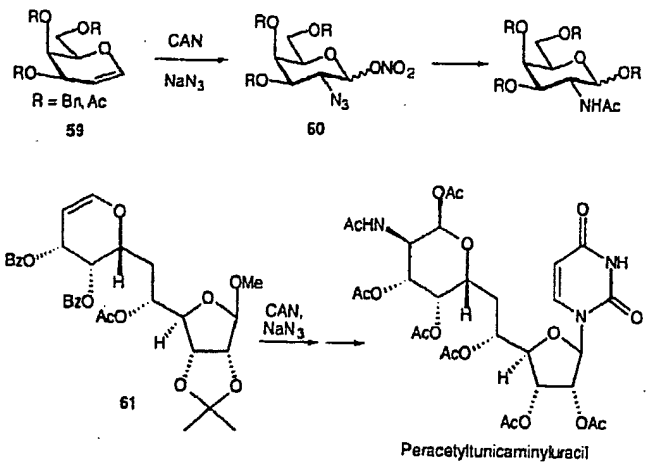
Given the excellent access to natural and unnatural glycals by either total synthesis or partial synthesis (see Section 2), it seemed appropriate to investigate the possibility of their use as precursors to glycosides of 2-acylaminosugars. Of course, in the light of the iodoglycosidation chemistry discussed in Section 4, the most obvious approach to introduce an equatorially disposed nitrogen at C2 would entail displacement of the axial iodo substituent (**55** → **56**, Scheme 19). However, S_N2 reactions at centers with (axial) leaving groups in a 1,2-*anti* relationship to an axial glycoside bond tend to be low yielding. A different approach to this problem for the case of an *N*-acetylmannosamine glycoside, developed in our laboratory, involved exploiting an intramolecular hydroxy nucleophile at C3 (**57** → **58**).[55] While interesting, this method is rather lengthy and, in any case does not address the critical series of glycosides



Scheme 19. Synthesis of an α-mannosamine from a glycal.

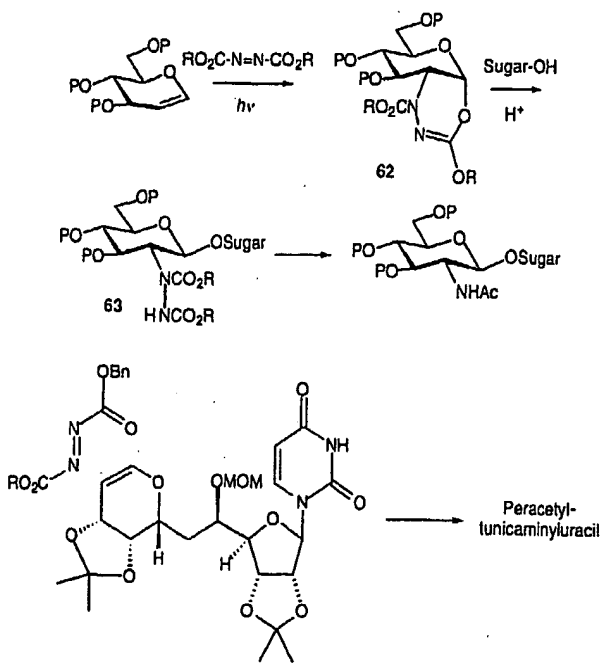
corresponding to *N*-acetylglucosamine. Such glycosides appear, for instance, in asparagine-linked glycoproteins (see Section 10).[58]

Two methods for introducing a nitrogen at C2 via a glycal had been studied earlier by Lemieux. An important first advance employed nitrosochlorination of glycals.[59] While this route constituted significant progress at the time, the methods to convert the oximino products to desired goal structures were not fully optimal as regards to yield and stereoselectivity. Better results were achieved by azidonitration (**59** → **60**, Scheme 20).[47] Indeed, this method led to the synthesis of various goal systems. For instance, in our first synthesis of tunicaminyluracil, we made recourse to azidonitration of glycal **61**, which had been derived from the LACDAC methodology.[29a]



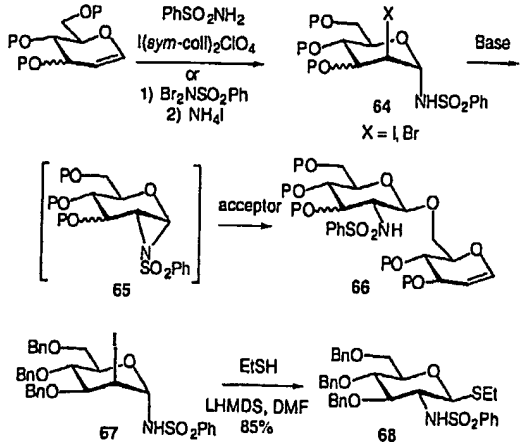
Scheme 20. Azidonitration of glycals. CAN = cerium ammonium nitrate.

A very interesting solution to the problem of converting glycals to such goal systems was developed by Fitzsimmons, Le Blanc, and co-workers.[60] It starts with the cycloaddition of azodicarboxylates with glycals, giving **62** (Scheme 21). Under various forms of acidic mediation, systems such as **62** can function as glycosyl donors (**62** → **63**). Several protocols were developed such that the hydrazodicarboxylate linkage can be reductively cleaved and the resulting products converted to desired 2-acetamido targets. Indeed, we took advantage of this chemistry in our second and more effective synthesis of tunicaminyluracil (Scheme 21).[29b]



Scheme 21. Cycloaddition of azodicarboxylates with glycals.

For reasons that will become clear when we discuss the total synthesis of allosamidin,[61] we sought a new method to reach glycal **66** (Scheme 22). The condition was that stereoelectronic factors rather than issues of local steric hindrance should govern product formation. In other words, application of the method to be developed to either a glucal or an allal bearing a C3 axial hydroxyl, or protected hydroxyl group, should result in the C2-α-acetamido product.

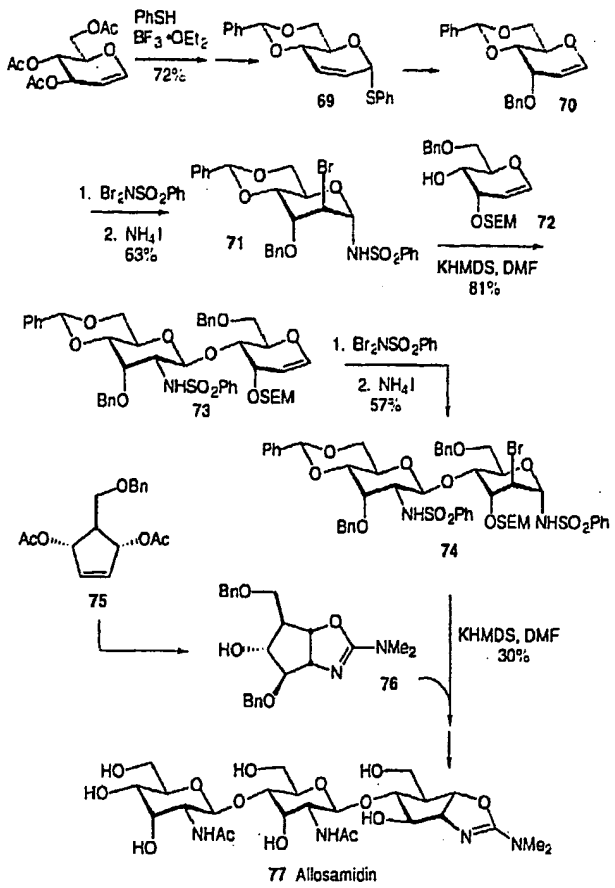


Scheme 22. Iodosulfonamidation of glycals. P = protecting group or sugar, LHMDS = lithium hexamethyldisilazide.

The key reaction developed by David Griffith to deal with this problem was that of sulfonamidoglycosylation.[62] The method, which involves *trans*-diaxial addition of an *N*-halobenzenesulfonamide to a glycal, leads to **64**. Under appropriate conditions, a range of nucleophiles can be used to convert **64** to glycosides of 2-α-benzenesulfonylglycosamine derivatives. The incoming acceptor can be a pyranose with a suitably differentiated hydroxyl group, or it can be a glycal (e.g. **66**) thus allowing for reiteration of the process. Also, the donor can be a di- or oligosaccharide terminating in a glycal linkage to undergo sulfonamidoglycosylation.

While we have not succeeded in properly characterizing the intermediate between the 2β-halo-1α-sulfonamidopyranosides (e.g. 64), and the product 66, we have reason to believe that the 1,2-sulfonylaziridine 65 is, in fact, the active glycosylating entity. This moiety functions as a very powerful electrophile, prompting clean β-attack by the nucleophile at the anomeric carbon. Several protocols have been developed for liberating the amino system from its 2-sulfonamide precursor (see Section 10). Furthermore, an iodosulfonamide can be readily converted to the corresponding ethyl thioglycoside (see 67 → 68, Scheme 22). The latter can subsequently be employed as an azaglycosyl donor to some advantage. This conversion has been proven to be very useful in several important cases where the direct glycosylation of iodosulfoamides fails (see Schemes 53 and 54).

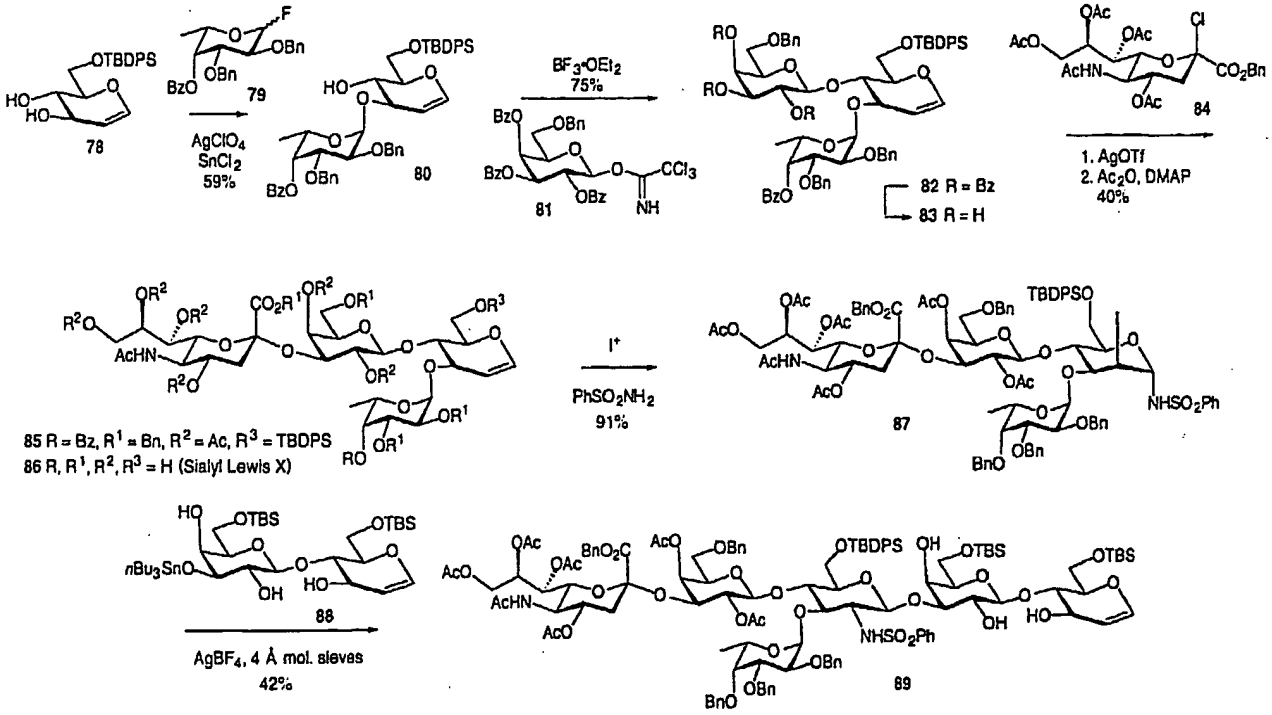
Below we describe three of the early applications of the sulfonamidoglycosylation of glycals. The first application of this new chemistry was in the total synthesis of the very powerful chitinase inhibitor allosamidin.[51] The route to allosamidin exploited sulfonamidoglycosylation at two stages. In the first application, the allal-type glycal 70 was used (Scheme 23). This glycal is available either from allose or, more interestingly, by rearrangement of the α-thiophenyl derivative 69 (in turn available by a sequence starting with the Ferrier transformation of D-glucal triacetate).[36] Bromosulfonamidation of 70 afforded 71, which was coupled to glycal 72 in the presence of potassium hexamethyldisilazide (KHMDS) to give 73. The latter then functioned as an azaglycosyl donor. Bromosulfonamidation of 73 afforded 74. Reaction of 74 with aglycone derivative 76 afforded, after several steps, allosamidin (77). Incidentally, the synthesis of 76 was accomplished by enantioselective desymmetrization of the *meso* compound 75,[64] thus enabling a pleasing route to the natural enantiomer.



Scheme 23. The synthesis of allosamidin. SEM = trimethylsilylethoxymethyl.

A second early application came in the important field of sialyl Le^x glycosides.[64–66] The sialyl Le^x substructure at the nonreducing end of glycoproteins on the cell surface is the key recognition element in E-selectin and P-selectin mediated adhesion.[67] This discovery has spurred interest in the field of sialyl Le^x chemistry.[68] The goals, as we defined them from a combined chemistry–biology perspective, focused on the synthesis of an intermediate that could serve as a launching point to reach various glycosides of sialyl Le^x. Such glycosides could be screened as potential inhibitors of the natural ligand.

Toward this end, we identified sialyl Le^x glycal derivative 85 as a suitable target (Scheme 24). We also hoped to study the



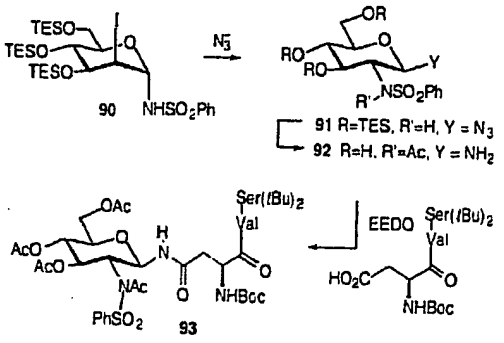
Scheme 24. Synthesis of a sialyl Le^x glycal.

properties of the fully deprotected derived glycal **86** in an ELAM binding assay. Moreover, if the azaglycosylation chemistry described above could be extended to the protected system **85**, we could gain access to a range of more extended oligomers from a late stage in the synthesis. In this way we would obviate the need for a separate lengthy synthesis for each assay candidate.

A key discovery in this regard, first registered by Jacquelyn Gervay and John Peterson, was that glycal **78**, containing silyl protection only at the primary (C6) hydroxyl group, undergoes selective fucosylation with **79** at the allylic alcohol center (C3) (Scheme 24). Conveniently, after fucosylation, the C4 hydroxyl was available to serve as the acceptor toward the galactosyl trichloroacetimidate donor **81**, thus affording Le^x derivative **82** (and, after deprotection, **83**). Sialylation of **83** with the known sialyl donor **84** and acetylation led to protected product **85**, and eventually to the deprotected sialyl Le^x glycal **86**.

Furthermore, **85** underwent iodosulfonamidation to afford **87** (Scheme 24). This compound served as an *N*-sulfonylglucosaminyl donor toward stannyl-activated glycosyl acceptors (e.g. **87** + **88** → **89**). The prospect of exploiting the glycal linkage for still further derivatization is obvious. A further simplification in reaching the Le^x glycal series employing a 1,2-anhydrogalactose derivative has been achieved, and this is described later in this review (see Scheme 50).[56]

The possibility of using a glycal linkage in the construction of glycopeptides by sulfonamidoglycosylation presented itself. In the event, iodosulfonamide **90** was treated with sodium azide to produce, cleanly, the anomeric β-azide **91**, which was converted to **92** (Scheme 25).[69] The anomeric amine was acylated by

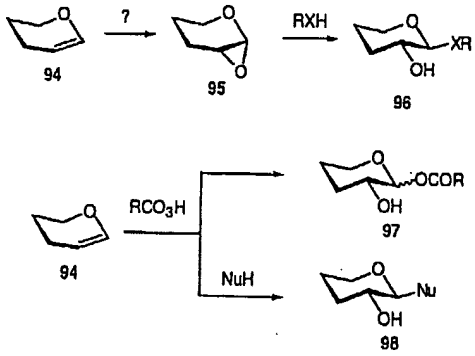


Scheme 25. Synthesis of N-linked glycopeptides from iodosulfonamides. TES = triethylsilyl.

suitable aspartate residues, including that of a tripeptide, using 2-ethoxy-*N*-ethoxycarbonyl-1,2-dihydroquinoline (EEDQ) to afford **93**. At that stage, deprotection of the sulfonamide in the presence of the sensitive glycopeptide bond, even at the level of a monosaccharide tripeptide was problematic. Given our rapidly developing methodology for the synthesis of oligosaccharides terminating in glycals (see Section 6), the ability to convert such compounds to asparagine-linked glycopeptides was seen to constitute an important goal in synthesis of large complex oligosaccharide–peptides. We shall return to the dramatic progress achieved in this area after addressing the matter of solid-phase oligosaccharide synthesis (see Schemes 73 and 74).

6. Applications of 1,2-Anhydrosugars to Glycoside Synthesis

While iodoglycosylation and sulfonamidoglycosylation provide valuable capabilities for the conversion of glycals to various glycosides, there was a need for a very general route to convert glycals into common glycosides of glucose, galactose, and mannose. Ideally the new methodology would embrace both α- and β-glycosides. In search of this type of method, we considered the possibility of directly converting glycals **94** to glycal epoxides **95** (Scheme 26). At the time we undertook this



Scheme 26. Direct epoxidation of glycals.

investigation, two serious impediments to the broad applicability of 1,2-anhydrosugars presented themselves. First, there had been no reported methodology for the direct conversion of a glycal to its 1,2-oxirane derivative. While such systems were well known for 60 years (for example Brigl's anhydride),[70] they had hitherto been prepared only after somewhat lengthy protocols from hexose derivatives. However, given our focus on glycals, we considered the possibility that such epoxides might be readily obtained directly from glycals. Previous attempts to prepare 1,2-anhydro systems from the reactions of various peracids with glycals led, not to the 1,2-oxiranes, but to products of their heterolysis (**97** and **98**).[71] Certainly, the indications were that glycal epoxides were exceedingly sensitive to opening of the oxirane ring by nucleophilic attack at the anomeric carbon.

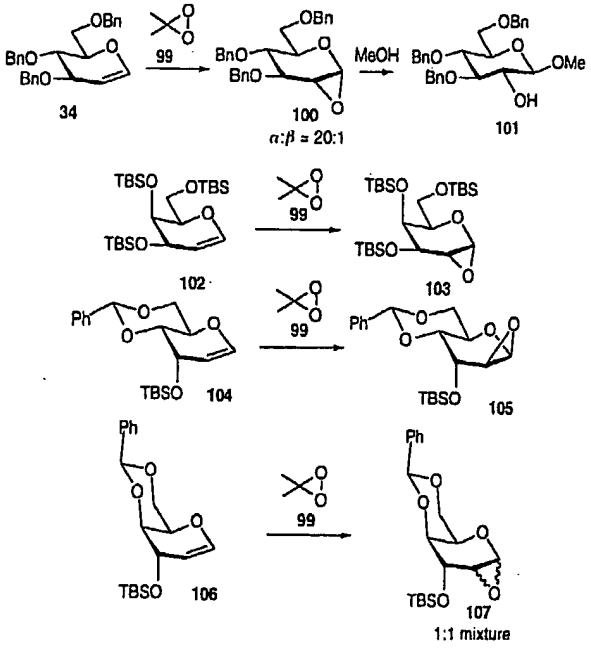
Also, the record of using α-epoxides such as **95** as stereoselective β-glycosylating agents for the formation of compounds such as **96** was none too promising.[72] Previous attempts to prepare disaccharides by employing a 1,2-oxirane as a glycosyl donor with various acceptors often resulted in nonstereoselective glycoside formation, although the application of such a donor in the historic Lemieux construction of sucrose[73] was certainly a milestone accomplishment in synthetic organic chemistry.

Owing to the difficulties associated with synthesizing 1,2-oxiranes, in most of their applications as glycosylating agents the systems contain acyl protecting groups at positions 2, 3 and 4. We wondered whether such resident groups might participate in the ring opening of the epoxide, thereby compromising clean inversion in the glycosidation reaction. In this connection, we were struck by reports from the laboratories of Schuerch and co-workers,[74] wherein perbenzylated 1,2-anhydroglucose underwent stereoregular polymerization under the influence of protic acids. It was recognized that in principle epoxy polymer-

ization of a 1,2-anhydrosugar corresponds to a reiterative glycosidation. If the polymer were indeed being produced in a stereodefined fashion with regular 1β linkages, it seemed that, at least in the polymerization case, the glycosylation reactions were stereospecific.

We therefore focused on several tasks. We sought a general method for the conversion of a glycal to a 1,2-anhydrosugar. The second emphasis was investigating whether glycals, which do not bear potential neighboring group participants of their resident functionalities, might serve as more stereoselective and efficacious donors in the formation of β-glycosides. Furthermore, we would investigate whether suitably differentiated glycals could function as glycosyl acceptors in glycosylations using glycal epoxide donors. We were not unmindful that should such a step be accomplished, the product would itself be a candidate for further elongation through reiteration of the sequence of epoxidation and coupling.

A major advance in our technology was contributed by Randall Halcomb. He found that a variety of glycals react smoothly with 2,2-dimethyldioxirane (99, DMDO),[75] prepared as a solution in dichloromethane according to the protocol of Murray.[76] For instance, glucal derivative 34 reacted smoothly with 99 to afford 100 in near-quantitative yield (Scheme 27). Solvolysis of 100 with neat methanol provided methyl glycoside 101, whose structure was confirmed unequivocally by NMR analysis. Based on configuration of the methanolysis products, we estimate the stereoselectivity of epoxidation to be at least 20:1 in favor of the α-isomer. We emphasize, however, that with resident acetyl protecting groups, the stereoselectivity of epoxidation is much reduced (cf. Scheme 46). Epoxidation of galactal derivative 102 provided 103.

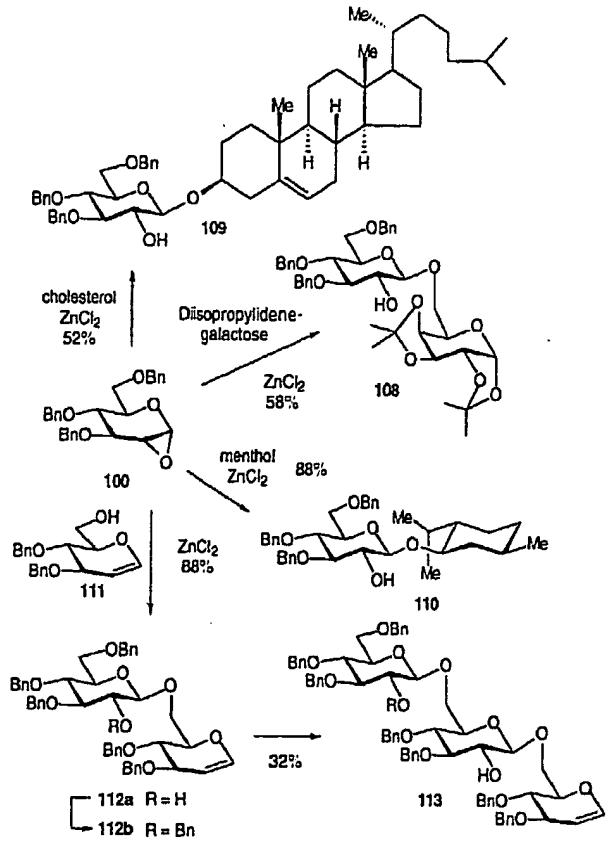


Scheme 27. Epoxidation of glycals by 2,2-dimethyldioxirane (99).

It was of interest to probe the allal series. In the event, glycal 104 bearing the axial 3-TBSO function undergoes quite selective epoxidation from its β-face, providing 105. On the other hand, the gulal derivative 106, with hindering substituents on both faces of the double bond, gave a 1:1 mixture of epoxides 107.

Armed with these results, we investigated the glycosyl-donating properties of these epoxides, particularly those derived from glucose and galactose. We soon discovered that the glycosylation of acceptors more complex than methanol and present to a roughly stoichiometric degree was slow and required promotion. A universal promoter has not been discovered. With moderately acidic acceptors such as phenols[77] and indoles[78] (vide infra) best results were obtained under basic conditions. Presumably under these conditions the kinetically active form of the acceptor is the alkoxide. With ordinary alcohol acceptors, including those in which the hydroxyl group is part of a saccharide, the most widely used promoter has been anhydrous zinc chloride. In some special applications (for example the synthesis of gangliosides) stannyl derivatives generated in situ gave the best results (vide infra).

Our earliest results with 100 as a donor are summarized in Scheme 28. Subsequent to these experiments, it was found that this particular oxirane is among the poorest of the donors[79, 80]

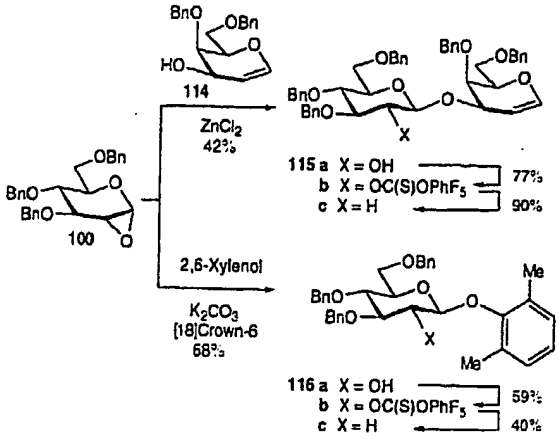


Scheme 28. Coupling of an anhydrosugar with representative acceptors.

and, depending on the case, some α-glycoside is produced. It has since been learned that glycosylation yields can be improved by constraining the C3 and C4 or C4 and C6 oxygen functions into a cyclic motif (see Schemes 37 and 80). Nonetheless, the method had already enabled an easily executed two-step pathway from glucal derivatives to β-glycosides. That these products are fashioned with a uniquely distinguished free hydroxyl group adjacent to the β-glycosidic bond became a crucial element in our synthesis of complex branched saponins and the blood group determinants (vide infra). Also, the finding that the glycosyl acceptor for the glycal epoxide can itself be a glycal (see formation of 112) had indeed established the basis for a reiterative

strategy for the synthesis of repeating β-glycosides such as **113**. This type of reiterability was in turn a central component of our solid-phase synthesis of oligosaccharides, in which the glycosyl donor is mounted to a solid matrix (see Section 10).

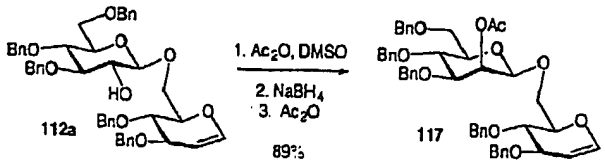
We also demonstrated the applicability of the glycal epoxide method to the synthesis of 2-deoxy-β-glycosides.[77, 81a] Here we took advantage of the uniquely placed free hydroxyl group at C2 as a target for deoxygenation. This was accomplished by free radical reduction of the derived pentafluorophenylthiocarbonate (Scheme 29).[81b] This demonstrates the feasibility of



Scheme 29. Coupling of epoxides with secondary alcohols and phenols as acceptors.

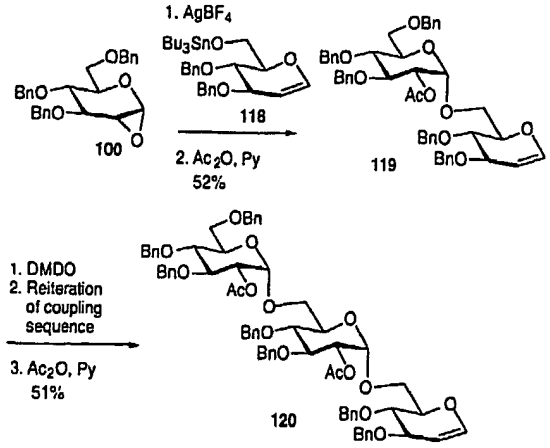
a Barton deoxygenation in the presence of a glycal linkage. Again, the basis for ready reiterability of the sequence is seen in the progression **100** + **114** → **115**. The glycosylation of 2,6-xylenol by donor **100** leading to **116a** set the stage for the synthesis of β-aryloxyglucosides and, following deoxygenation, the corresponding 2-deoxyaryloxyglucosides such as **116c**.

The uniquely generated 2'-hydroxyl group arising from opening of the 1,2-epoxide donor has also been exploited in the synthesis of β-mannosides by means of the Garegg oxidation/reduction protocol (Scheme 30).[82] Thus, oxidation of **112a** with acetic anhydride and DMSO followed by reduction of the unpurified product with $NaBH_4$ and acetylation provided selectively the β-mannoside **117** in 89% yield.



Scheme 30. Creation of β-mannosides by oxidation/reduction.

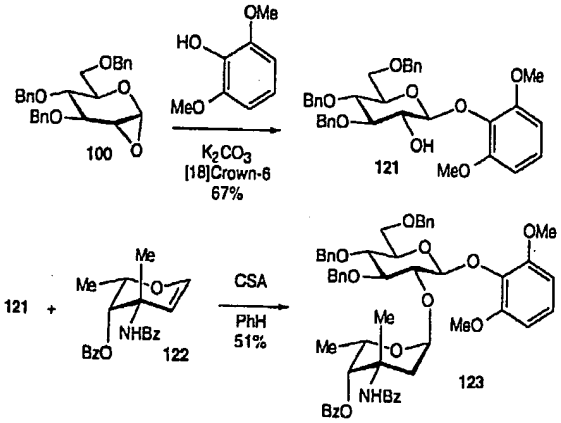
In addition, we have shown that in certain cases an α-glucoside can be obtained directly from a glucal epoxide (Scheme 31).[83] Reaction of the epoxide **100** with stannylether **118**, promoted by $AgBF_4$, followed by acetylation afforded the α-glycoside **119**. The process was readily reiterated to provide the trisaccharide **120**. Reactions with secondary acceptors proceeded in lower yields and exhibited sharply diminished stereoselectivities. This method is presently limited to primary

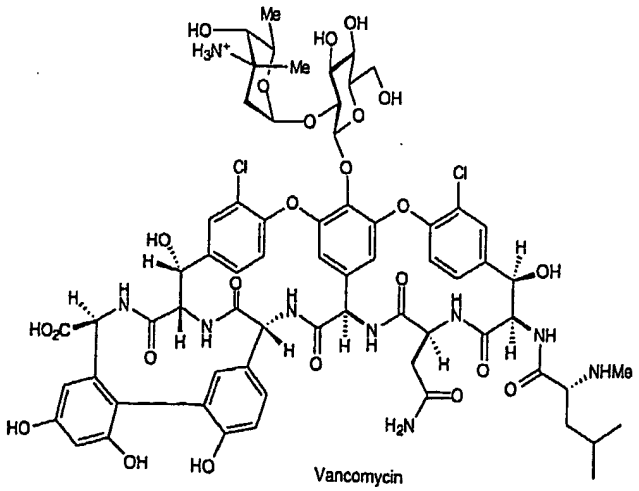


Scheme 31. Direct formation of α-linked glucose linkages. Py = pyridine, DMDO = dimethyldioxirane (**99**).

hydroxyl acceptors and certainly does not constitute a comprehensive solution to the challenging problem of generating α-glycosides. A solution to the conversion of glucals to α-glucosides was attained by transformation of the α-epoxides to known α-glucoside donors (see Scheme 40).

In Scheme 32 we demonstrate the applicability of the glycal epoxide method to the facile construction of a complex 2-branched β-aryl glycoside.[84] Compound **121** was constructed from **100** and 2,6-dimethoxyphenol. In the original approach **123** was to be prepared by iodinative glycosylation of **121** and **122** followed by reduction. As matters transpired the one-step

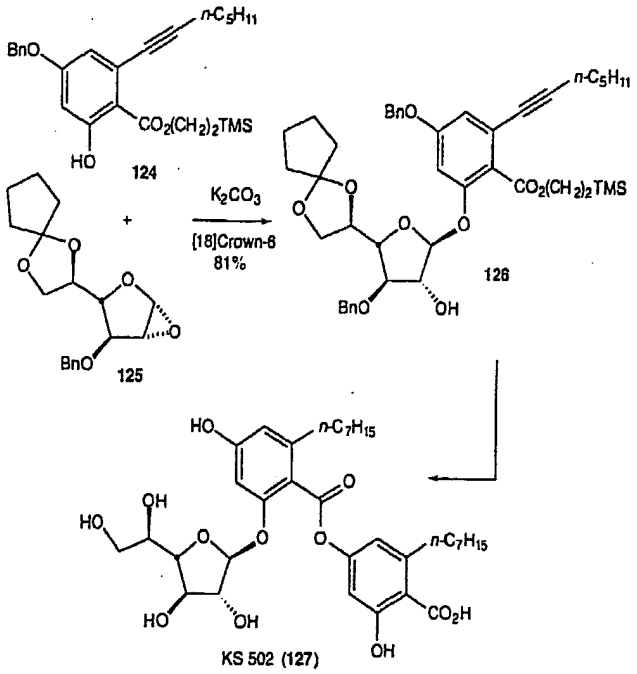




Scheme 32. Synthesis of a branched β-aryl disaccharide. CSA = 1-(*S*)-(+)-camphorsulfonic acid.

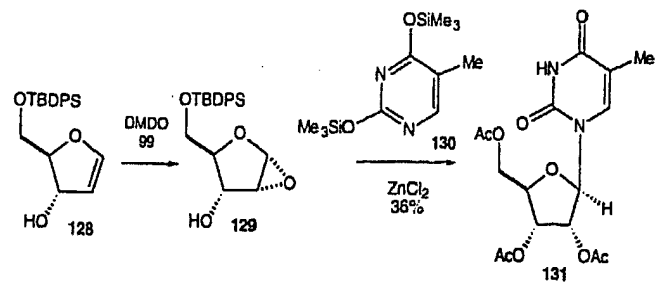
proton-mediated coupling of **121** and **122** proceeded stereospecifically. We note that product **122** encapsulates the salient features of the branched aryl glycoside domain of the potent antibiotic vancomycin.

It was of interest to attempt to extend these ideas to the synthesis of β-glycosides of furanose derivatives. Russel Dushin took up this possibility in the context of the first total synthesis of the calmodulin-dependent phosphodiesterase inhibitor KS 502 (**127**, Scheme 33).[85] The salicylate glycosyl acceptor **124** was assembled in a straightforward way. The furanoid 1,2-glycal epoxide **125** was synthesized from D-talonic acid via the corresponding glycal. Coupling of **124** and **125** gave **126** with high stereoselectivity. The steps from **126** to KS 502 (**127**), while not trivial, proved to be manageable.



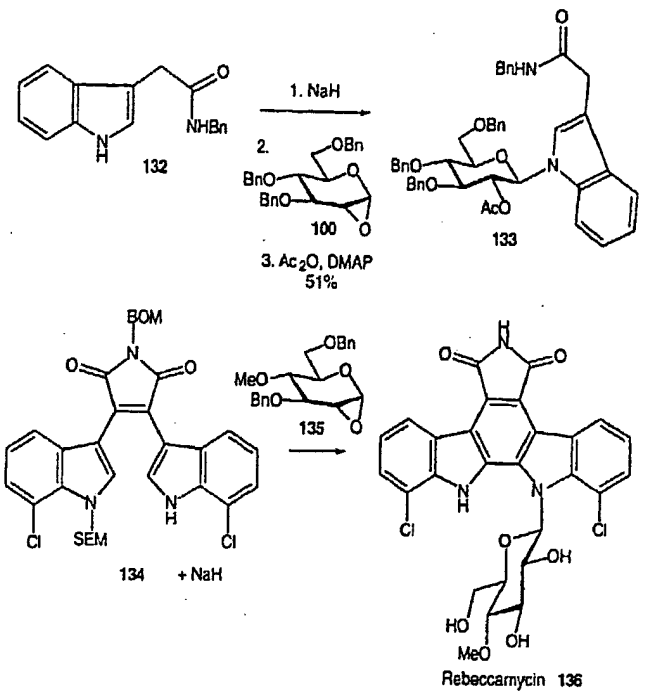
Scheme 33. Synthesis of KS 502 (**127**).

The possibility of utilizing 1,2-oxiranes derived from furanoid epoxides in the synthesis of nucleosides was briefly examined.[86] Interestingly, the furanoid glycal **128** underwent smooth epoxidation with dimethyldioxirane to produce, in 9:1 selectivity, the α-epoxide **129** (Scheme 34). Thus, at least in the furanoid glycal series, "hydroxyl direction" in the epoxidation seems to be operative. Interesting in this regard is the recent report of Murray which demonstrates that in nonpolar solvents the epoxidation of 2-cyclohexen-1-ol with 2,2-dimethyldioxirane does exhibit directivity.[87] In our case, the labile **129** was used as a glycosyl donor in a Vorbrüggen-type reaction[88] with pyrimidine derivative **130** to give stereospecifically **131**. The yield for fashioning of the nucleoside bond is somewhat disappointing and has not yet been improved.



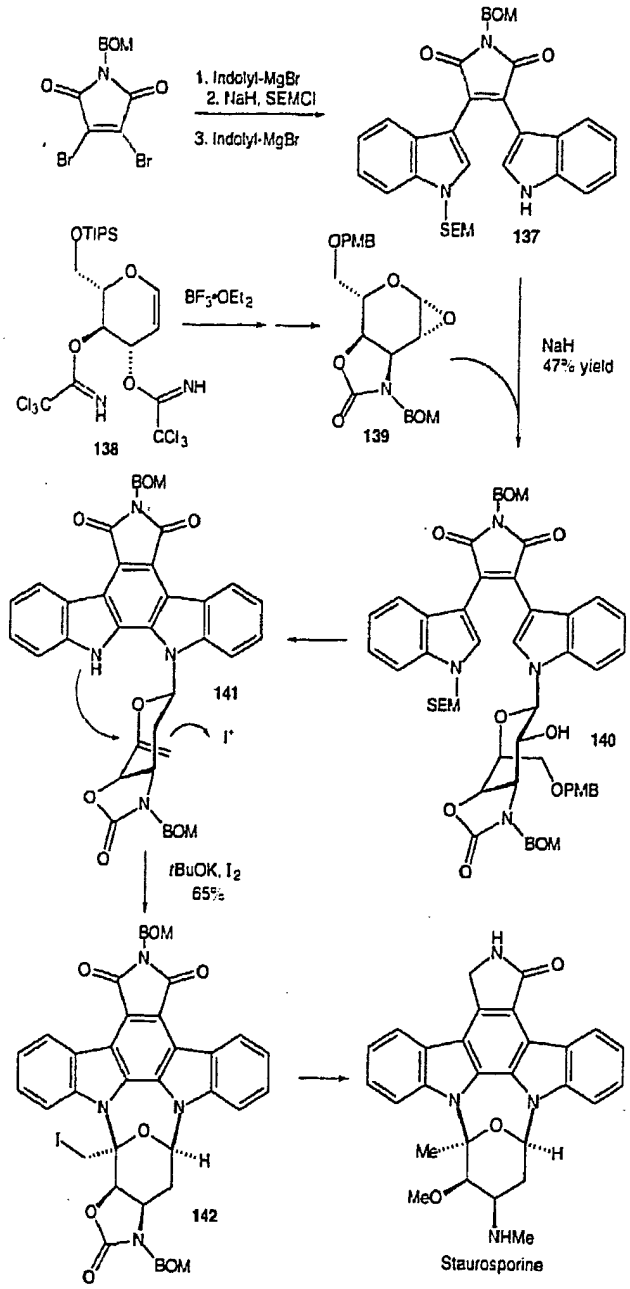
Scheme 34. Synthesis of nucleoside 131 (d.r. = 4:1).

Another area of inquiry was the possibility of using glycal epoxides to glycosylate indoles. Michel Gallant took up this problem. He found that deprotonation of **132** with sodium hydride followed by glycosylation with **100** and acetylation gave rise to model system **133** (Scheme 35).[78] Our motivation in studying the N-glycosylation of indoles with such oxiranes was the potential application of such reactions to the synthesis of natural products. Gallant and J. T. Link first brought our newfound capability to bear in the synthesis of the antitumor agent rebeccamycin (**136**). They took recourse to differentiated secoimide **134** as the acceptor and the differentiated oxirane **135** as the donor.

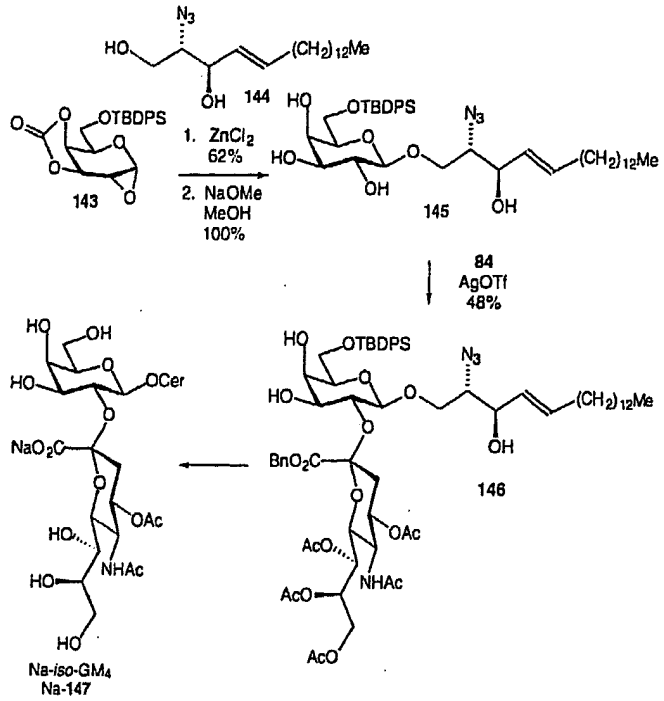


Scheme 35. Indole glycosylation by anhydrosugars. DMAP = 4-dimethylaminopyridine.

A more adventurous, yet related project was apparent: a total synthesis of the premier protein kinase-C inhibitor, staurosporine. Link, Subha Raghavan, and Gallant rose to the challenge. They noted the possibility that staurosporine might be constructed by formation of two indolyl glycoside bonds. Accordingly, the aglycone **137** and glycosylating agent **139** were assembled (Scheme 36).[89,90] The former was assembled from *N*-benzyloxymethyl 3,4-dibromomalemide and indolylmagnesium bromide through the modular motif previously used in the rebeccamycin effort. The synthesis of the hexose portion started with the mono(triisopropylsilyl) (mono-TIPS) protected L-glucal, which was converted to bis(trichloroacetimidate) **138**. A rather interesting vinylogous Schmidt glycosylation was employed, in which the "leaving group" is the trichloroacetimidate function at C3 "donor" carbon. This led, eventually, to the 1,2-epoxy donor **139**. Coupling of the sodium salt of **137** with



Scheme 36. The synthesis of staurosporine. BOM = benzyloxymethyl.



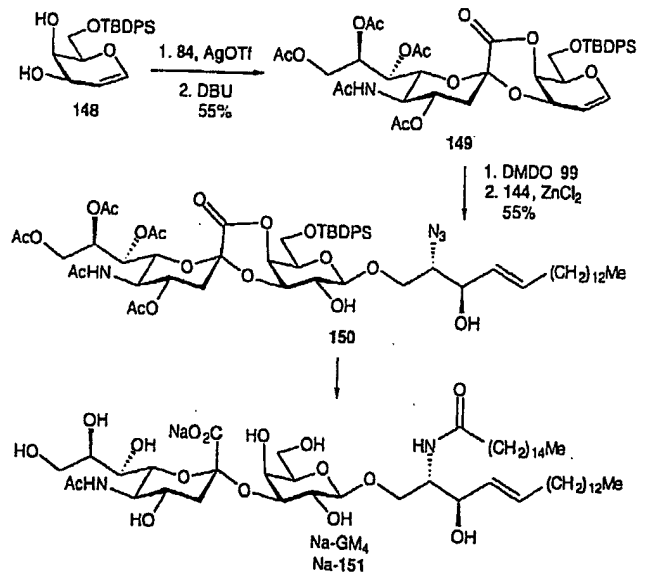
Scheme 37. Synthesis of "iso-GM_4." Cer = ceramidyl.

139 afforded 140. Several further steps yielded 141, the potassium salt of which underwent smooth intramolecular "indolo"-N-glycosylation to afford 142 and, after a number of steps, staurosporine was obtained.

The possibility of using glycal epoxides in the construction of gangliosides was examined.[91, 92] An important advance in this area relied on the use of galactal-derived epoxide 143 (Scheme 37). Previously, in many cases, galactal-derived epoxides did not function well as stereospecific β-galactoside donors. The use of a cyclic carbonate protecting group engaging the C3 and C4 oxygens (see structure 143) can lead to favored β-galactosylation in a variety of situations. In the event, reaction of Schmidt construct 144 with 143 resulted in a high degree of selectivity for β-glycoside formation at the primary alcohol. Cleavage of the carbonate gave rise to tetraol 145. Contrary to many apparent precedents, 145 underwent sialylation at the C2 rather than the C3 hydroxyl group. This surprise was uncovered when the product, later known to be 146, was carried through several steps to produce 147, whose spectroscopic properties were inconsistent with formulation as GM_4. We have referred to 147 as "iso-GM_4."

Even today we are unable to explain this remarkable departure from the precedents of Hasegawa et al.[93] and our own experience in the sialyl Le^x series (see Scheme 24, formation of 85).[66] It must be a special consequence of the presence of the pre-ceramide side chain, although the particular functional group responsible for an apparent activation at C2 cannot be specified.

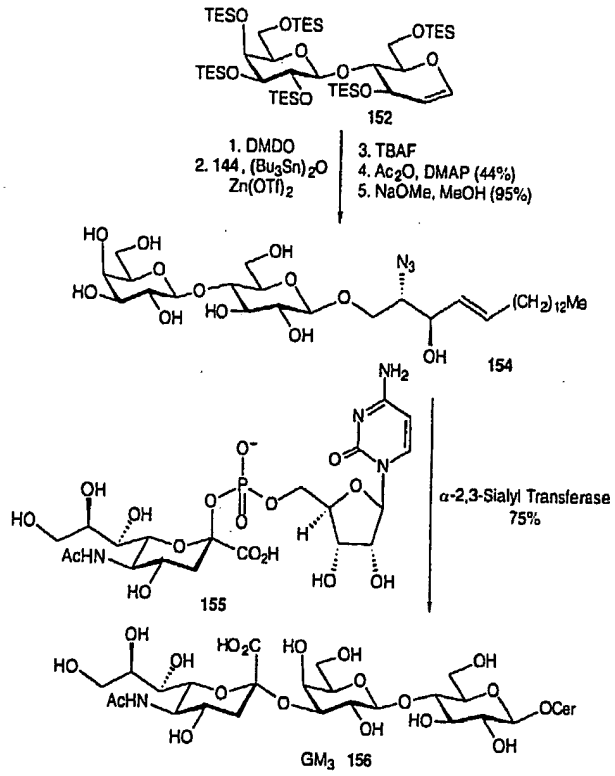
Fortunately, however, a straightforward way of circumventing this difficulty presented itself to Gervay and Peterson. Thus, glycal 148 was directly submitted to sialylation with 84 (Scheme 38). Coupling occurred smoothly at the allylic hydroxyl group, generating a 3-sialylated galactal derivative which, upon treatment with 1,8-diazabicyclo[5.4.0.]undec-7-ene



Scheme 38. Synthesis of GM_4.

(DBU), provided the 3,4-spirolactone, glycal 149. Epoxidation with dimethyldioxirane and glycosylation with ceramide precursor 144 produced glycoside 150. This compound was, indeed, converted to the pure sodium salt of GM_4 (151).

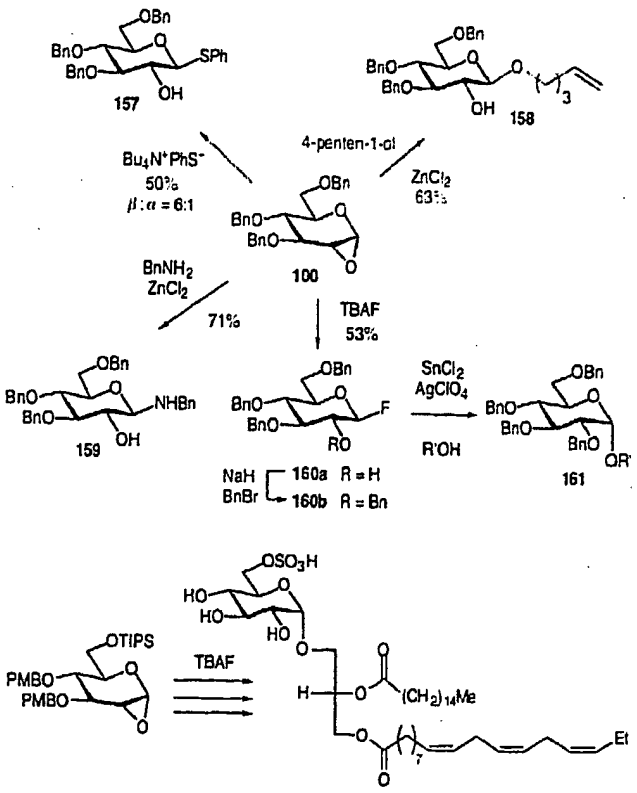
A combination of chemical and biological methods was used by Kevin K. C. Liu in a particularly straightforward synthesis of the important ganglioside GM_3 (156).[91] Thus, lactal was uniformly protected with triethylsilyl groups to produce 152 (Scheme 39). The latter underwent epoxidation and coupling



Scheme 39. Synthesis of GM_3.

with the stannylated version of the Schmidt diol 144, followed by desilylation to produce, in high yield and high specificity, compound 154. This compound responded to enzymatic sialylation with cytidine monophosphate (CMP) sialic acid (155) under mediation by a 2,3-sialyl transferase. After total deprotection, GM_3 (156) was in hand. Though cofactors were not regenerated in our experiments, this chemoenzymatic approach provides the most direct route to GM_3.

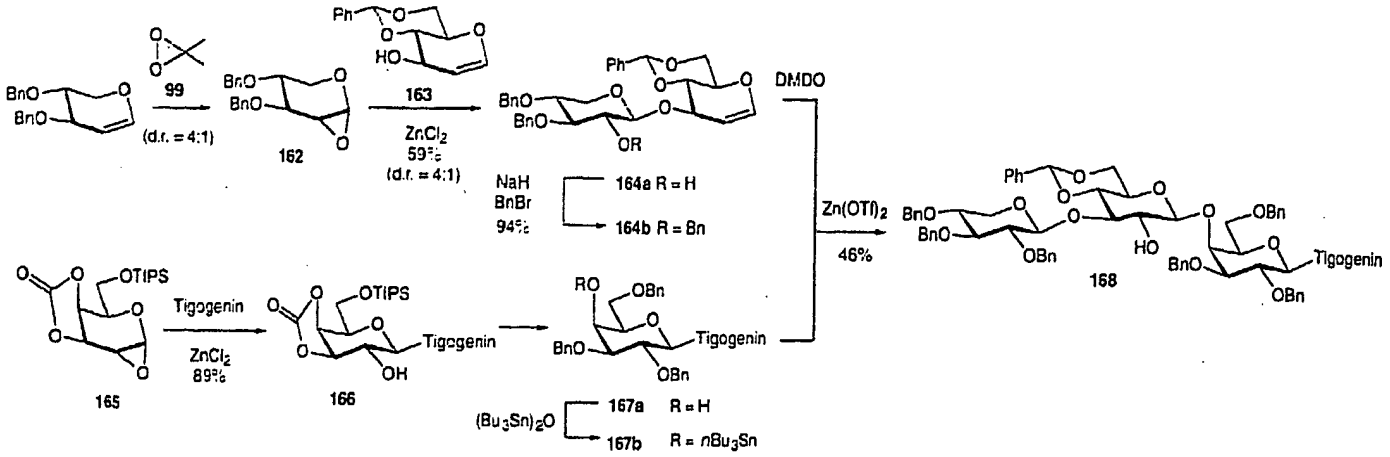
Thus far we have focused on the use of glycal epoxides in the synthesis of β-glycosides. It is also possible to convert such epoxides to other glycosylating agents (Scheme 40).[94] For instance, Dana Gordon found that compound 100 could be converted to the thiophenyl glycoside 157, the pentenyl glycoside 158, the benzylaminoglucoside 159, and fluoroglycoside 160. Compound 160a, upon benzylation of the single free hydroxyl group at C2, gave rise to 160b, which served as a glycosyl donor in a conventional Mukaiyama reaction[95] to produce α-glycoside 161. The route from glycals to α-glycosides found important application in Gordon's synthesis of the cyanobacterial sulfolipid, a compound alleged to have anti-HIV activity (Scheme 40).[96] Still another application arose during our synthesis of the carbohydrate section of acarbose.[97]



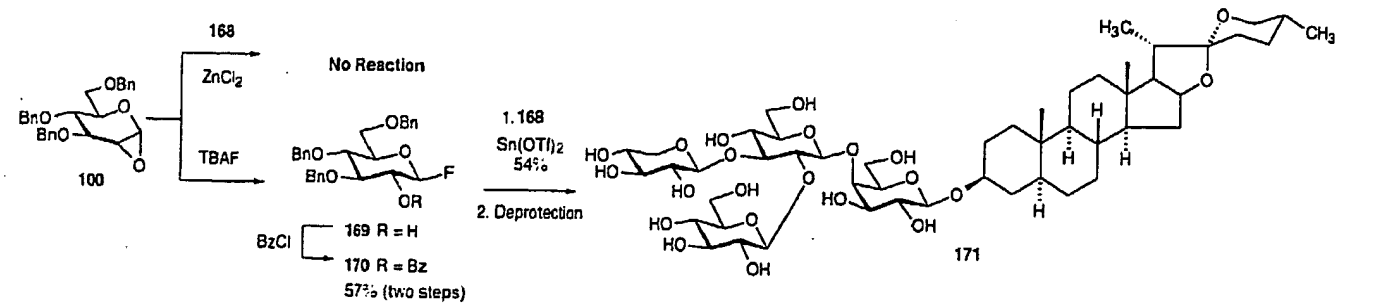
Scheme 40. Conversion of anhydrosugars to other donors, and synthesis of a cyanobacterial sulfolipid.

At this stage we could perceive the emergence of a comprehensive strategy for oligosaccharide construction based on glycal acceptors and glycal-derived donors. We began to field-test the concepts of glycal assembly in the context of specific biologically active and important goal systems. It is almost indisputable that the "combat worthiness" of new concepts in synthesis and new synthetic methodology are best evaluated in the context of multifaceted target structures (for example natural products), where unanticipated difficulties are apt to arise.

An advance in the development of the logic of glycal assembly was achieved in our synthesis of the complex saponin, desgalactotigonin (Schemes 41 and 42).[98,99] John Randolph took note of the collection of β-glycoside linkages in this target. In particular, we focused on the branched glucose ring in the carbohydrate sector, which is β-linked to the axial hydroxyl group at C4 of a galactose. Particularly intriguing is the branching of this glucose: its C2-hydroxyl is β-linked to another glucose, while at C3 it is β-linked to a xylose. The introduction of this central glucose ring in the form of glycal derivative 163 brought forth major simplifications in protecting group strategy. The free hydroxyl at C3 served as an acceptor toward the xylal-derived epoxide 162 to afford, after benzylation, 164b. The acceptor for coupling to this donor was fashioned from galactal cyclic carbonate derivative 165. Epoxidation of 165 followed by coupling to the tigogenin aglycone afforded 166. Once again this type of epoxide had served us very well as a β-galactosylating agent (cf. 143 in Scheme 37). Several steps were required on the resultant glycoside: 1) cleavage of the cyclic carbonate, 2) engagement of the 4- and 6-hydroxyls in a benzylidene protecting group, 3) benzylation at C2, 4) cleavage of the benzylidene linkage, and 5) rebenzylation at C6 and stannylation at C4 to produce acceptor 167b. Coupling of 167b with the epoxide of 164b under



Scheme 41. Synthesis of desgalactotigonin (part 1).



Scheme 42. Synthesis of desgalactotigonin (part 2).

mediation by zinc triflate afforded, albeit in only 46% yield, the steroidal trisaccharide **168**. The glycal epoxide coupling method had targeted a unique hydroxyl group at C2 of the central glucose for branching.

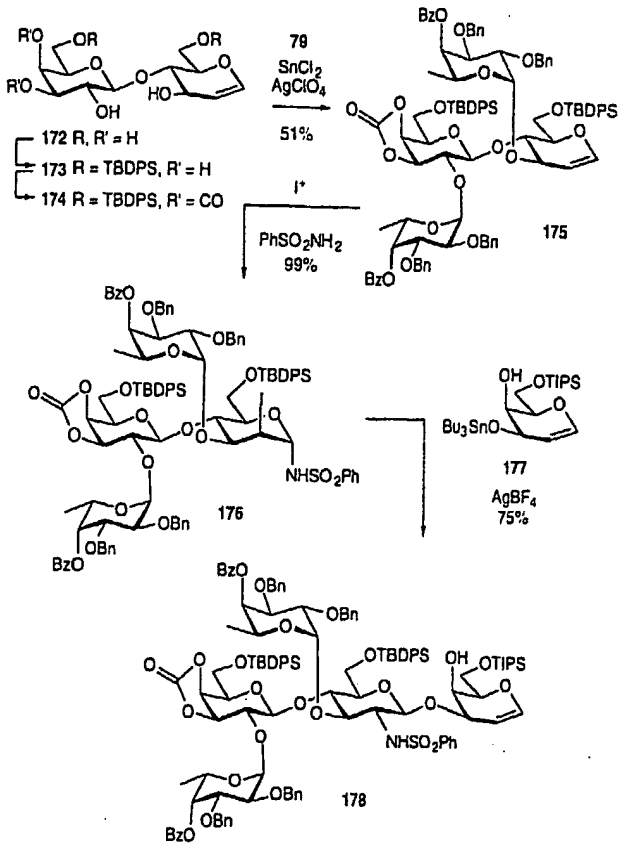
At this stage, however, the free hydroxyl group in **168**, flanked as it was by glycosidic bonds at C1 and C3, did not lend itself to glycosylation with epoxide donors (cf. **100**, Scheme 42). However, we were readily able to fashion a competent donor from **100**. Thus, fluoridolysis[94] afforded **169**, which was converted to its benzoate **170**. The latter functioned with apparent α-face participation to heavily favor β-glycoside formation. Indeed, coupling of **170** with **168** was smoothly accomplished, and was followed by deprotection to provide desgalactotigonin (**171**).

In summary, the logic of glycal assembly had allowed for a high degree of convergence in assembling the complex branched target system. Glycal epoxide opening had been used to expose a unique hydroxyl group to function as a glycoside acceptor (see **169**) and to install a participating neighboring group in a regiospecific fashion. The participating group could be used to direct a fluoroglycosyl donor toward β-glycoside formation (en route to **171**).

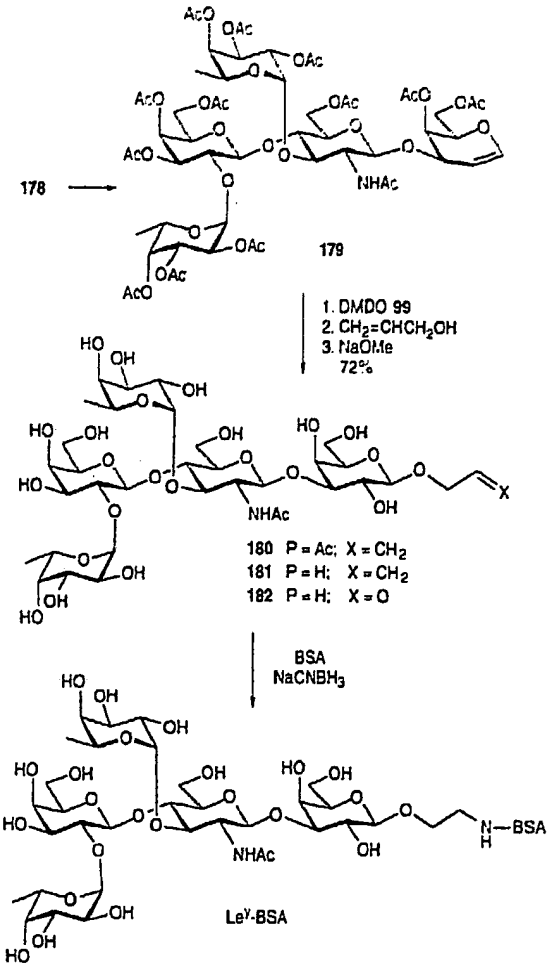
7. Lewis Determinants, Blood Group Determinants, and Tumor Antigens

The grounds seemed to be sufficiently secure to apply the glycal assembly logic to the synthesis of Lewis and blood group determinants. In so doing we would be drawing from glycal epoxides and halosulfonamides in the same synthesis. The glycal epoxide methodology would be used to install β-glycosidic linkages and the azaglycosylation methodology would be used to incorporate the *N*-acetyl glucosamine substructure. John Randolph and Victor Behar took up this problem in earnest. In the synthesis of the first target, the Le^y determinant,[100, 101] the objective was not only to prepare the carbohydrate sector of the determinant, but also to conjugate it to a carrier protein such that it would more closely approximate the realities in biological systems. The Le^y determinant was of particular interest to us because it had been previously identified as an important epitope for eliciting antibodies against colon and liver adenocarcinoma cell lines.[102] It has recently been implicated as a marker in metastatic prostate cancer.[103] We hoped to simulate this capacity with fully synthetically derived antigen. For this purpose it would be necessary to conjugate this synthetic product to a carrier of the type used to stimulate immune response. To preserve the integrity of the core epitope sector, it would be insulated from the carrier through a spacer domain.

Inspection of the Le^y structure points toward the possibility of building from a central lactose core for this purpose. Given our preference for exploring the chemistry of glycals, lactal (**172**) was identified as the lactose equivalent (Scheme 43). The two primary hydroxyl groups were silylated to produce the bis-TBDPS derivative **173** (TBDPS = *tert*-butyldiphenylsilyl). At this point we took advantage of the *cis* relationship of C3′ and C4′ of **173** by engaging these hydroxyl substituents in the form of cyclic carbonate **174**. Thus, the required two hydroxyl groups at C3 and C2′ of the galactose moiety were readily designated in two steps to function as glycosyl acceptor sites. Fluorosugar **79** was employed as the glycosyl donor. It bears a nonparticipatory benzyl ether at C2 and a potentially participating benzoate



Scheme 43. Synthesis of a Le^y glycal.



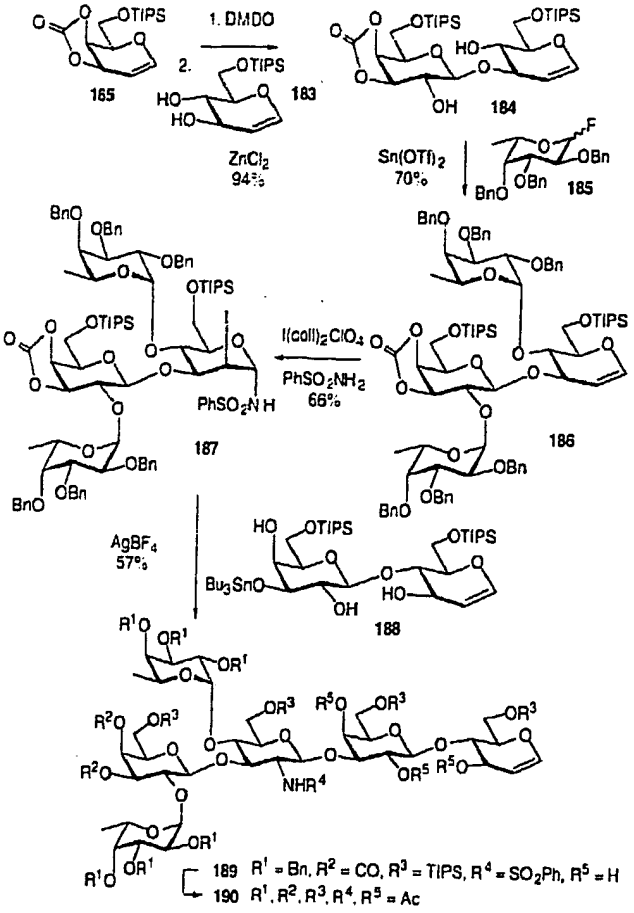
Scheme 44. Conjugation of Le^y to a protein carrier.

at C4. In the event, treatment of **174** with **79** under suitable conditions results in clean incorporation of two fucose residues with formation of compound **175**. The glycal double bond was then subjected to iodosulfonamidation under the usual conditions to give rise to **176**, which functioned as a masked azoglycosyl donor. The critical coupling of **176** with the mono-TIPS stannylated galactal derivative **177** gave the bisfucosylated compound **178** in 75% yield. Thus the tetrasaccharide determinant was efficiently constructed such that it still contained an exploitable double bond.

Before we proceeded to the final glycosylation, it was strategically useful to convert the benzenesulfonamide function to an acetamido group as well as to regularize all of the protecting groups as acetates (Scheme 44). This goal was accomplished and led to compound **179**, which was subjected to the action of dimethyldioxirane. The epoxide thus produced functioned as a competent donor with allyl alcohol as the acceptor to provide **180**. Removal of all acetate groups, affording **181**, was accomplished by the action of sodium methoxide. Thus the logic of glycal assembly combined with the powerful technologies of azaglycosylation and of glycosylation via glycal-derived 1,2-anhydrosugars allowed for a highly concise and convergent synthesis of **181**. We were then ready for the conjugation phase and relied on the reductive amination method developed Bernstein and Hall in a much simpler context.[104] In practice, the double bond of the allyl group of **181** was cleaved to give the uncharacterized aldehyde **182**. The latter was conjugated to bovine serum albumin (BSA) through the action of sodium cyanoborohydride. Amino acid and carbohydrate analysis of the pseudo-glycoprotein indicated the incorporation of approximately 15 pentasaccharide units into the 38 lysine residues theoretically available. Immunization studies of conjugates of **182** in mice are currently in progress, and the earliest results are promising.

It is possible that oligosaccharide domains will someday be synthesized from non-carbohydrate building blocks. At the present time, however, this prospect is virtually unimaginable. Therefore, the basic challenges of synthetic design lie in selecting the molecular components of the synthesis in such a fashion that their assembly in the desired sense is least complicated. Conciseness is the goal in the orchestration of the diverse functionalities and in chemically identifying those loci destined to serve as the glycosyl acceptors and donors in the coupling steps. In both the syntheses of **171** and **181** the use of glycals toward these goals had been demonstrated.

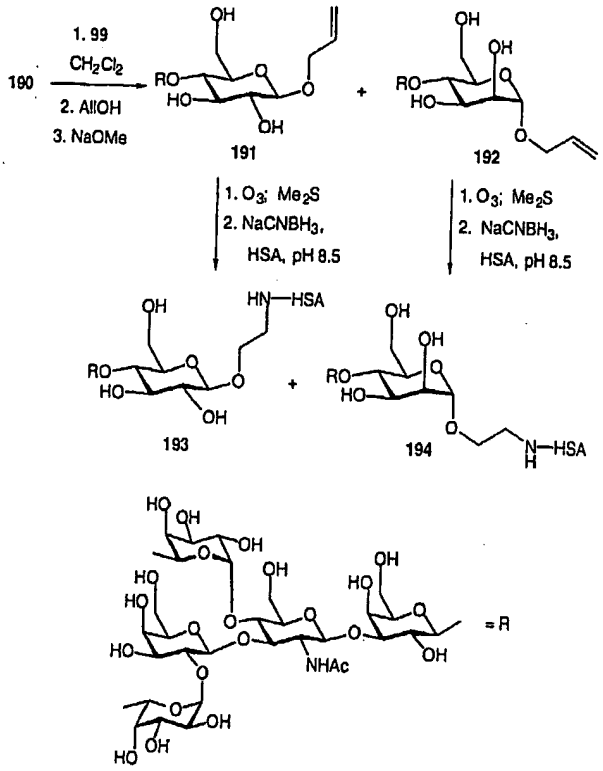
John Randolph accomplished the synthesis of the Le^b determinant, again in bioconjugatable form.[101, 105] This determinant is more challenging from the synthetic standpoint than the Le^y target, because lactal (**172**), trivially available from lactose, cannot serve as a starting material. We proceeded as follows: the TIPS galactal derivative **165** was converted to its epoxide through the agency of dimethyldioxirane (Scheme 45). The acceptor **183** was readily fashioned from glucal itself. Here we could take advantage of another enormous simplification of the glycal method. Coupling of **183** with the epoxide of **165** occurred exclusively at the C3 hydroxyl of **183** to provide **184**, in which the particular hydroxyls to be fucosylated (at C4 of the glucose and C2' of the galactose) have been smoothly distin-



Scheme 45. Synthesis of a Le^b glycal.

guished from other functions. Twofold fucosylation did, indeed, occur using the readily available donor **185** and the tetrasaccharide glycal **186** was efficiently obtained. Compound **186** was prepared for azaglycosylation through iodobenzenesulfonamidation to afford **187**. The latter reacted with lactal derivative **188**, which was prepared from lactal (**172**) in two steps. Coupling of **188** and **187** provided **189**. Thus, the critical tetrasaccharide recognition domain of the Le^b determinant had been assembled in a highly convergent fashion and insulated, through a disaccharide spacer, from the implement to be used in bioconjugation. For this purpose, all silyl groups were removed through the action of tetra-*n*-butylammonium fluoride (TBAF) and the benzyl groups were cleaved by sodium in ammonia. The crude product was peracetylated to give **190**, which was converted to its 1,2-anhydrosugar derivative, as a mixture of isomers, and thence to the corresponding allyl glycosides (Scheme 46). This serious breakdown in the stereochemistry of epoxidation arose from the presence of a resident acetate protecting group in the terminal glucose residue. As described earlier (see Scheme 26), triacetyl glucal is also a poor substrate for stereoselective epoxidation. Since conjugation occurs at some distance from the epitope domain, it is not clear that the stereochemistry of the glycoside bond leading to the carrier domain is of consequence at the biological level.

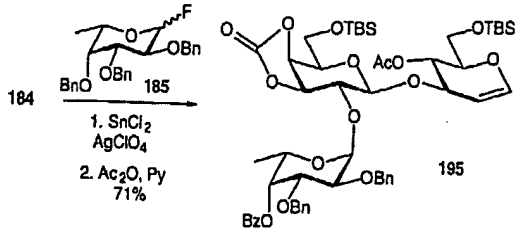
Cleavage of all acetate groups gave rise to **191** and **192**. These oligosaccharides were separately ozonolized and the resultant glycolic aldehyde products were reductively coupled to human serum albumin (HSA) by the Bernstein–Hall protocol[104] to provide adducts **193** and **194**.



Scheme 46. Conjugation of Le^b to a carrier protein. All = allyl.

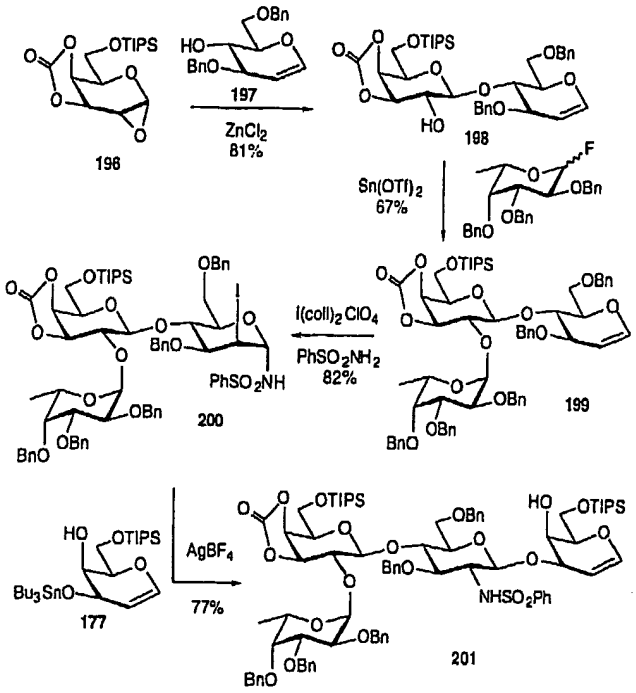
Approximately 33 carbohydrate hexamer units were incorporated, presumably by linkage to side chain amino groups of lysine. This construct is of particular interest to us because it incorporates the recognition domain implicated in the binding of *Helicobacter pylori* to gastric epithelial cells.[106] This form of infection is claimed to be one of the major causative elements of gastric ulcer and possibly gastric cancer. The possibility of synthesizing soluble binding agents for *H. pylori* constitutes an exciting goal for the glycal assembly methodology.

The chemistry described here can readily be accommodated into a synthesis of an H-type I tetrasaccharide as well.[101] This is accomplished by a selective α-fucosylation of the disaccharide unit **184** (Scheme 47). Fucosylation with donor **185**, as before, occured with a 5.5:1 selectivity at the 2′-hydroxyl in preference to reaction at the 4-hydroxyl, providing **195**.



Scheme 47. Synthesis of an H-type I glycal.

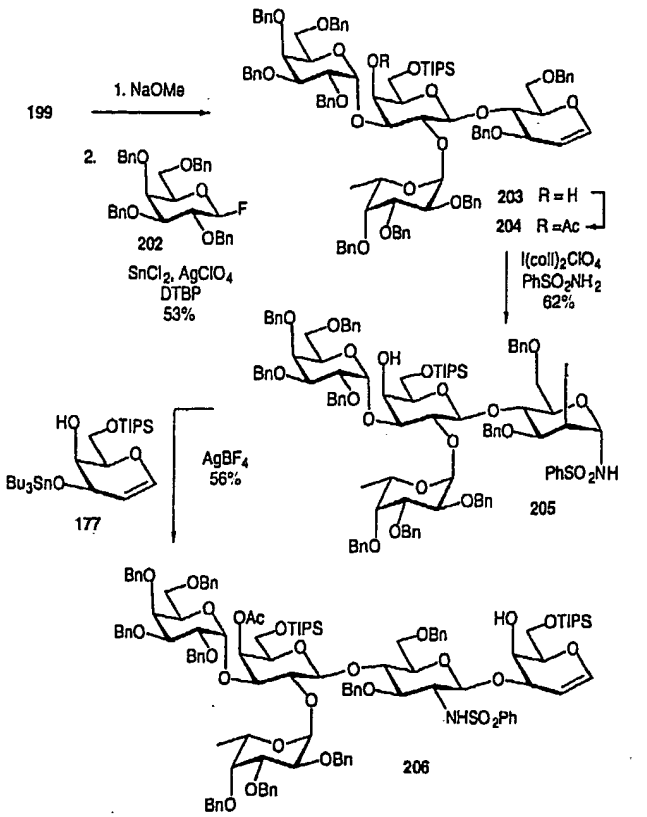
Furthermore, we have synthesized a tetrasaccharide having the H-type II structural domain (Scheme 48).[101] Reaction of epoxide **196** (derived from **165**) with the glucal **197** provided the differentiated lactal **198**, in which the newly presented C2′-hydroxyl is available for the requisite fucosylation. $Sn(OTf)_2$-promoted coupling yielded trisaccharide **199** bearing the H-type II domain. We further demonstrated that the structure



Scheme 48. Synthesis of an H-type II glycal.

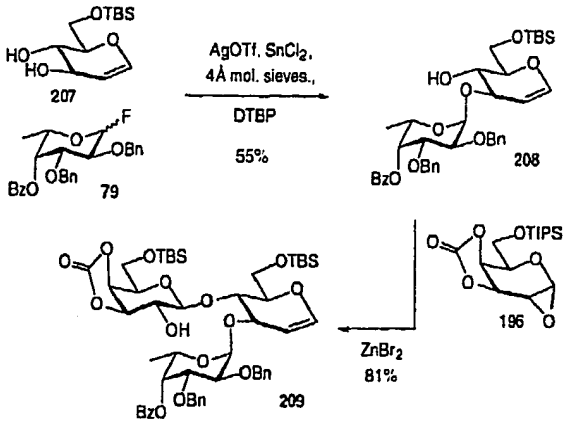
can be extended to a tetrasaccharide, employing the standard iodosulfonamide chemistry, to provide **200**. The latter, upon reaction with **177**, afforded **201**.

We have extended glycal chemistry toward the synthesis of the human A and B blood group determinants, which govern the ABO typing system. The synthesis of the core structure of the B determinant is shown in Scheme 49.[107] In the described case, trisaccharide **199** (Scheme 48) with the H-type II domain was used as the starting point. The carbonate was cleaved and the resulting diol was glycosylated with fluorosugar **202** to provide tetrasaccharide **203** containing the B-type domain. After acetylation, standard iodosulfonamide chemistry was employed to provide **205**. This B-type II structure was extended to the pentasaccharide **206** by utilizing the standard coupling conditions with acceptor **177**. The pentasaccharide **206** was readily deblocked to provide the unprotected version of the compound. Considerable progress in the A group series has also been accomplished at this writing, but the project is not yet complete (see Section 11).[107]



Scheme 49. Synthesis of a B-type II glycal. DTBP = di-*tert*-butylperoxide.

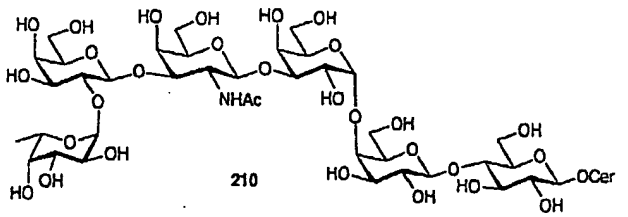
Returning to the series of Lewis determinants, our synthesis of the Le^x domain, previously described in Scheme 24, was greatly simplified by the use of a 1,2-anhydrosugar donor (Scheme 50).[66] Fucosylation of monoprotected glucal **207** afforded the disaccharide **208**, which is analogous to the acceptor employed in the earlier synthesis (cf. **80** in Scheme 24). In this later case, however, the acceptor was found to react under optimized conditions with the epoxide **196** to give Le^x trisaccharide **209** in 81 % yield. Epoxide **196** is far more accessible than donor **81** was in the earlier work.



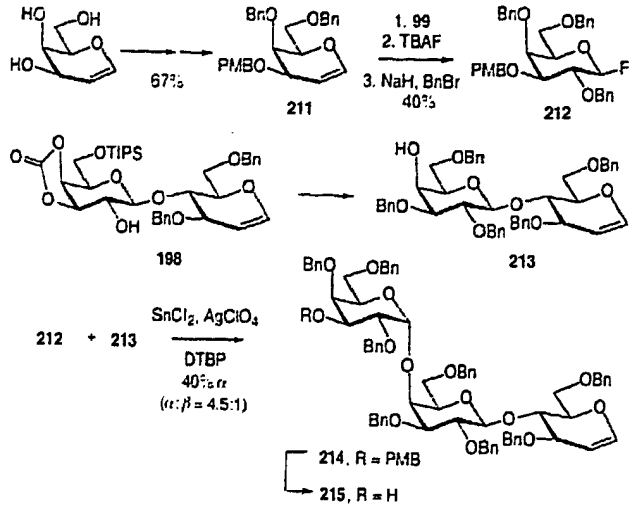
Scheme 50. A concise synthesis of the Le^x domain (cf. Scheme 24, compound 82).

8. Total Synthesis of the Breast Tumor Antigen: Challenges and Solutions

The glycal assembly method has culminated in the recently completed synthesis of a hexasaccharide glycosphingolipid, which is a breast tumor associated antigen of potential clinical importance. Compound **210** was isolated from breast cancer cell line MCF-7 and was immunocharacterized by the monoclonal antibody MBr1 (Scheme 51).[108] Our synthesis of **210** involved the construction of two trisaccharide domains, which were then brought together to provide the hexasaccharide.[109] Galactal **211** was converted into the fluorosugar **212** (Scheme 52). The desired acceptor **213** was fashioned from disaccharide **190** (itself



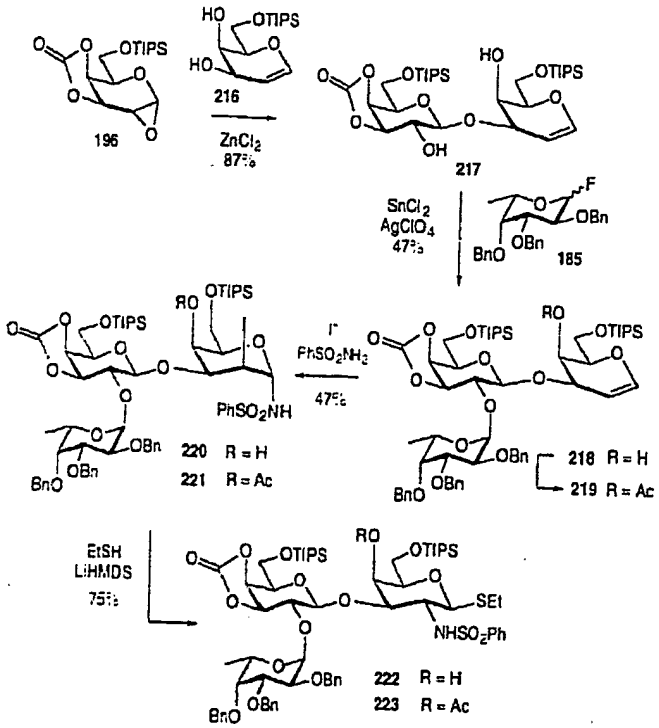
Scheme 51. Structure of the MBr1 carbohydrate antigen.



Scheme 52. Synthesis of a trisaccharide acceptor.

obtained by glycal coupling, see Scheme 49) after protecting group manipulations. Coupling of **212** and **213** afforded the trisaccharide **214**. Deprotection of the PMB ether provided **215**, setting the stage for merger with a suitable trisaccharide donor.

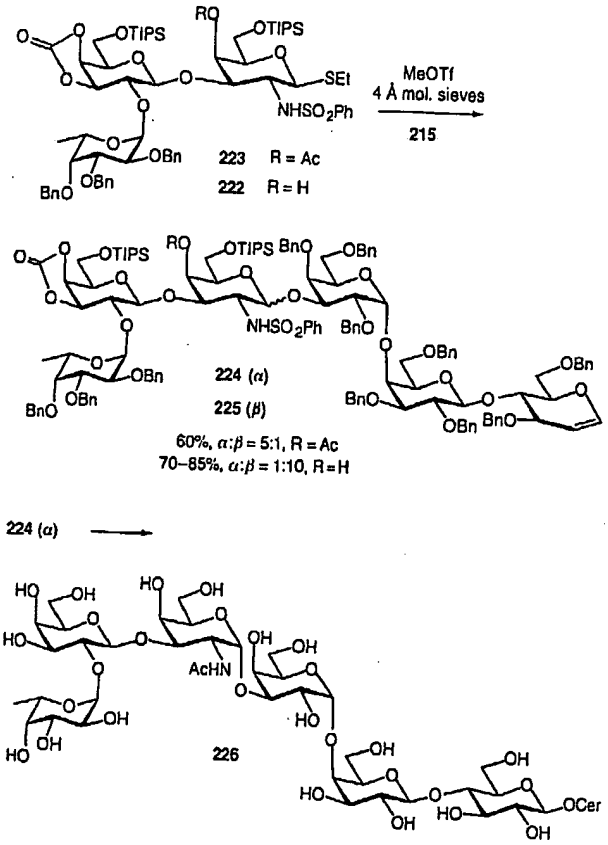
Construction of the donor began with **196**, which was glycosylated with acceptor **216** to afford the disaccharide **217** with excellent selectivity (Scheme 53). Regioselective fucosylation of the equatorial hydroxyl of **217** with donor **185** provided the trisaccharide **218**. This trisaccharide was acetylated to produce **219** which, after now standard treatments, was transformed to iodosulfonamide **221**. Unfortunately, iodosulfonamide donors of this type were not competent in the desired direct coupling reaction (see Scheme 22) with various trisaccharide acceptors. A large excess of difficultly available acceptor would be necessary, and this requirement is certainly not appropriate. We therefore



Scheme 53. Synthesis of a trisaccharide donor. LiHDMS = lithium hexamethyldisilazide.

examined the conversion of **221** to thioglycosides donors (see Scheme 22). Treatment of iodosulfonamide **221** with lithium ethanethiolate indeed afforded exclusively the β-ethyl thioglycoside **223**. Such compounds, under promotion by methyl triflate, function as azaglycosyl donors in coupling reactons with even complex donors. (In Section 11 we discuss some limitations in the projected merger of highly hindered partners.)

Precedent established in our program had suggested that, in coupling reactions, donors of this type would give the β-configurated product, presumably due to sulfonamide participation.[110] In the event, the reaction of **223** with the acceptor **215** afforded a hexasaccharide, which was advanced through the remaining manipulations in the synthesis (Scheme 54). However, the spectral properties of the ultimate product **226** did not

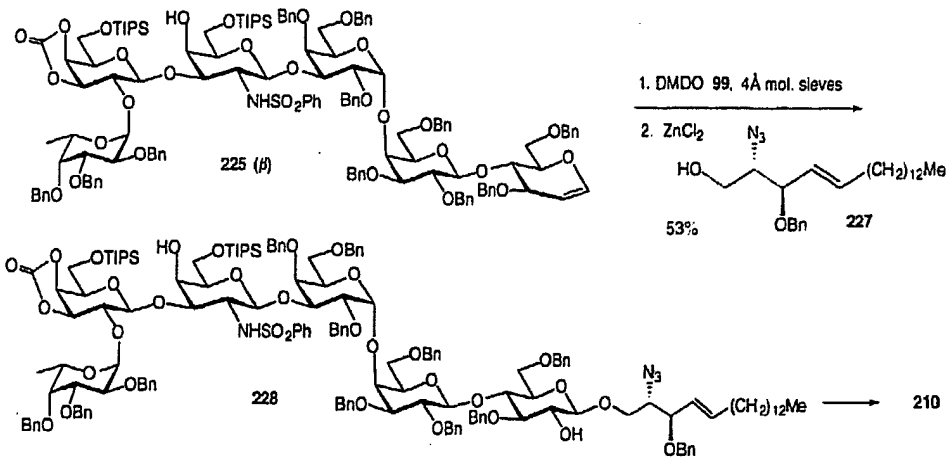


Scheme 54. Synthesis of the hexasaccharide glycal **226**.

correspond to those reported by Hakomori for the natural antigen, assumed to be properly represented by structure **210**. On this basis, and on the basis of self-consistent spectral analysis, we concluded that the material obtained from the coupling of the two trisaccharides had arisen from selective formation of the unexpected (and undesired) α-linked product **224**. We subsequently found that when the trisaccharide donor **222** (obtained by the sequence **218** → **220** → **222**) was employed in the key coupling reaction, the desired β-configurated product **225** was indeed obtained with high selectivity. Thus, there may be an unsuspected electronic or participatory effect biasing the system towards formation of the α-linked product when the 4-hydroxyl group is substituted . Alternatively, there may be a positive β-directing effect exerted by the 4-hydroxyl group of the donor. We emphasize that the occurrence of α-glycosylation and the remarkable turnover in selectivity has not yet been fully general-

ized, though it has been observed in several other cases (see synthesis of asialo-GM_1 in Section 11). This matter is being currently investigated and should be codified in due course. It does serve to once again underline the subtlety of the influences on the stereochemistry of glycosylation. The outcome is not merely a function of the type of donor and the type of reaction conditions employed. In complicated cases it can be much influenced by specific molecular interactions between donor and acceptor.

The properly configured hexasaccharide **225** was epoxidized and coupled with ceramide precursor **227** to provide **228** (Scheme 55). This ceramide attachment can be conducted more

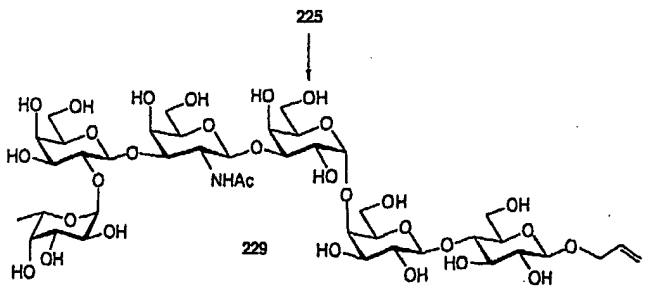


Scheme 55. Completion of the synthesis of the MBr1 antigen.

efficiently on trisaccharide **214**. For reasons that are not yet clear, this alternate route, which is being investigated, presently gives a lower β/α ratio in the (3 + 3) coupling when the acceptor already contains the lipid fragment at the reducing end.

Compound **228** was elaborated and deprotected to afford the natural material **210**. The fully synthetic antigen **210** has been shown to bind to monoclonal antibody MBr1 in the enzyme linked immunosorbent assay (ELISA) and in immune thin layer chromatography assays, while the unnatural isomer **226** exhibits very weak binding in the same assays. Also, MBr1 is strongly reactive with human breast cancer cell line MCF-7 by flow cytometry. Preincubation of MBr1 with glycosphingolipid **210** completely inhibits this reactivity with MCF-7.

Hexasaccharide **225** was also converted to the corresponding allyl glycoside **229** and through this to protein conjugates, as described previously for Le^b and Le^y (Scheme 56). Early studies indicate that our synthetic constructs are immunogenic in vivo. The usefulnesss of the antibodies thus produced against cancer



Scheme 56. Formation of an MBr1 allyl glycoside for protein conjugation.

cells is currently being evaluated (see Section 11). The ultimate goal is to develop compounds suited for vaccinelike applications in cancer treatment.

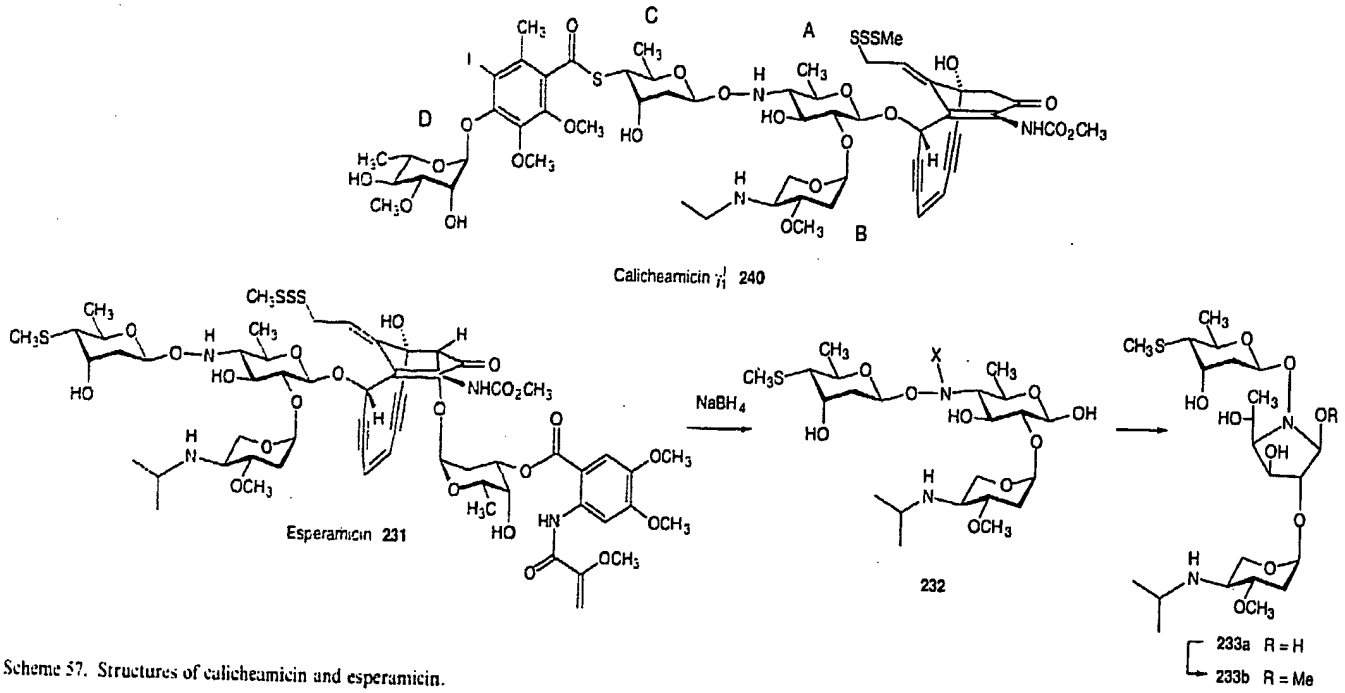
9. The Calicheamicin Problem

In the examples of glycal assembly discussed thus far ensembles were created from familiar carbohydrate building blocks. One of the large advantages of the glycal assembly method has been a reduction in the number of protecting group manipulations necessary to bring about the synthesis of complex target structures. A challenging extension of the glycal assembly method, in which several of the building blocks were far from conventional, was in the construction of the carbohydrate domains of the enediyne antibiotics calicheamicin (**230**, Scheme 57) and esperamicin (**231**).[111-115]

These investigations had several goals. First, we wanted to synthesize these sugar domains so that their biological properties could be examined in the absence of the effector feature of the drugs. Since the intact carbohydrate domains had not been successfully retrieved by degradation of the drugs, this objective seemed to be attainable only by synthesis. The realization of this synthesis through glycal assembly chemistry would confront us with some fresh challenges not encountered with conventional carbohydrate domains.

Moreover, we hoped to accomplish a total synthesis of calicheamicin γ_1^I itself. We had previously synthesized the aglycone domain, calicheamicinone, in racemic as well as enantiomerically pure form.[116] For a total synthesis it was our goal to deliver a fully synthetic carbohydrate domain, as a glycosyl donor, to an appropriate aglycone construct functioning as the acceptor. It seemed likely that the viability of the glycosyl donor would require extensive protecting group manipulations for the hydroxyl, alkylamino, and hydroxylamino functions. Thus, it would be crucial that deprotection of the carbohydrate domain be feasible with survival of this and the aglycone domain. Correspondingly, it would be desirable if the aglycone sector were in a maximally advanced state when functioning as the acceptor. In that way, the burden of dealing with the myriad of sensitive functionality subsequent to coupling would be minimized.

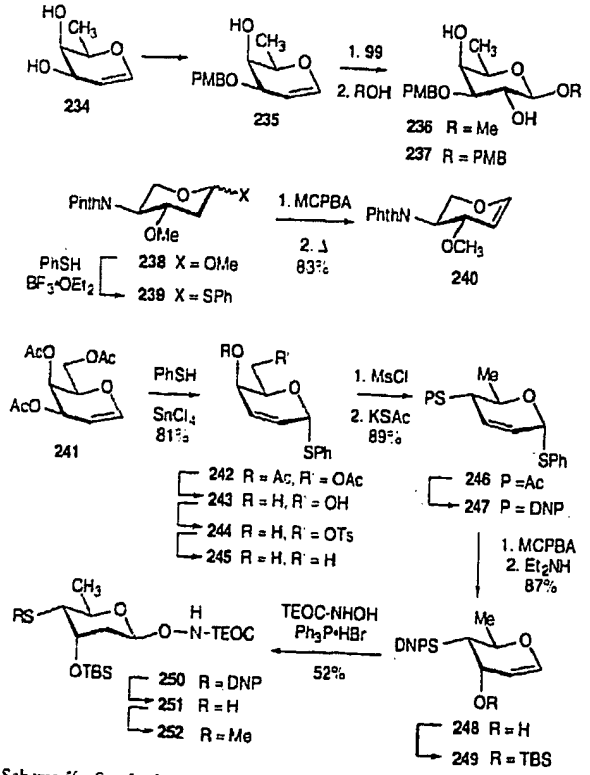
With these goals in mind, we started work on the core carbohydrate domain of esperamicin (**231**). Chemists at Bristol Myers Squibb with access to the drug had found that treatment of **231** with sodium borohydride led to **233a**, in addition to uncharacterized aglycone product (Scheme 57).[117] This reaction implied that reduction of the drug was producing **232** en route to **233a**, which was characterized as methyl glycoside **233b**. Obviously, a perception of the approximate rate of this rearrangement was of great interest to us. Presumably, our eventual car-



Scheme 57. Structures of calicheamicin and esperamicin.

bohydrate sector glycosyl donor (in the calicheamicin series) would be fashioned from 233a through manipulations at a free hydroxyl group at the anomeric carbon of its reducing end. The Bristol Myers result suggested that the concurrent presence of a free reducing end and a free NH group on the hydroxylamino spacer might well be incompatible. If this restriction were indeed the case, protection of the latter would be necessary as we passed through a construct with a free reducing end.

For the synthesis of the esperamicin carbohydrate domain, three glycals were mobilized (Scheme 58). Thus, D-fucal (234) was readily converted to 235. The second glycal, 240, was prepared from the previously reported methyl glycoside 238 via the anomeric thiophenyl compound 239 by pyrolysis of its derived sulfoxide.



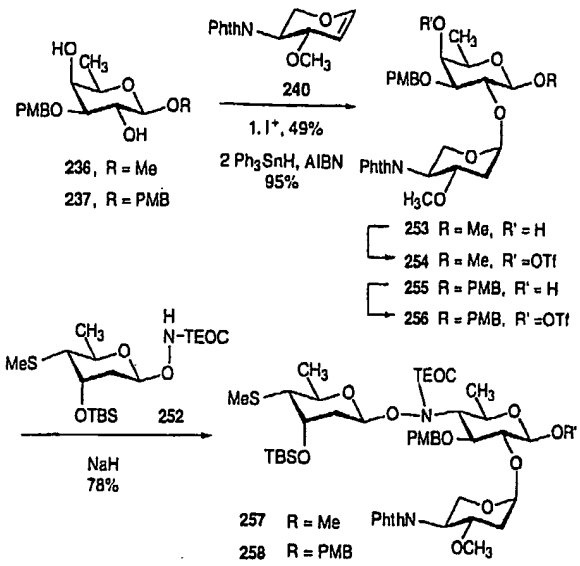
Scheme 58. Synthesis of the esperamicin carbohydrate domain (part 1). MCPBA = *m*-chloroperbenzoic acid. Ms = methanesulfonyl. Phth = phthaloyl. Ts = toluenesulfonyl.

The fashioning of the required thiosugar-containing glycal was challenging. D-galactal triacetate 241 was converted to 242 by thiophenyl Ferrier rearrangement.[36] After formation of diol 243 and thence tosylate 244, reduction afforded 245 and soon thereafter 246 and 247. Following rearrangement of the derived sulfoxide and suitable protection, 249 was in hand. This compound served as a donor with *N*-trimethylsilylethyloxycarbonyl (TEOC) hydroxylamine to afford 250. The chemoselectivity of the addition reaction was only 1.5:1 (the minor product was the *N*-glycoside). The stereoselectivity, however, was very high in favor of the β-glycoside. Presumably this exclusive β-face attack is a consequence of the resident α-axial-OTBS function (TBS = *tert*-butyldimethylsilyl). Removal of the dinitrophenyl (DNP) group and methylation provided 252.

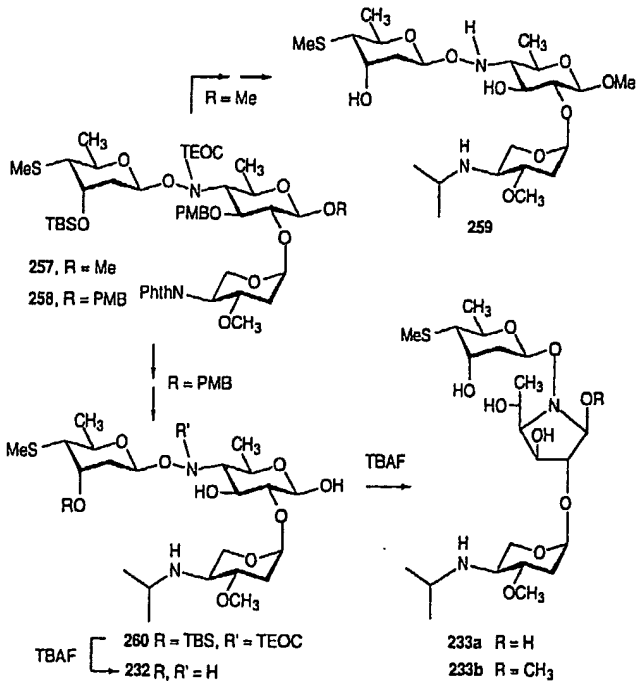
The assembly process started with epoxidation of 235 along conventional lines. Reaction of the resultant anhydrosugar with methanol and alternatively *p*-methoxybenzyl alcohol afforded 236 and 237, respectively (Scheme 59). Iodoglycosylation of 240 with 236 and 237 occurred quite selectively at the C2 (equatorial) hydroxyl to afford, after deiodination, disaccharides 253 and 255, respectively. Thus, the epoxide opening and iodoglycosylation strategies had been choreographed to provide rapid routes to the unusual disaccharides 253 and 255. These compounds were in turn converted to triflates 254 and 256, which were smoothly coupled to the carbamate sodium salt derived by deprotonation of 252.[118] In this way, tricyclic compounds 257 and 258 were in hand.

In the methyl glycoside series (257) the phthalimide was cleaved and an *N*-isopropylamine linkage fashioned by reductive amination with acetone. Cleavage of the TEOC and TBS protecting groups afforded methyl glycoside 259 (Scheme 60).

Similarly, in the *p*-methoxybenzyl (PMB) case (258), once again the phthalimide was cleaved and the *N*-isopropyl group introduced. We next exposed the free reducing end by cleaving



Scheme 59. Synthesis of the esperamicin carbohydrate domain (part 2). AIBN = α,α'-azobisisobutyronitrile. Tf = trifluoromethanesulfonyl.

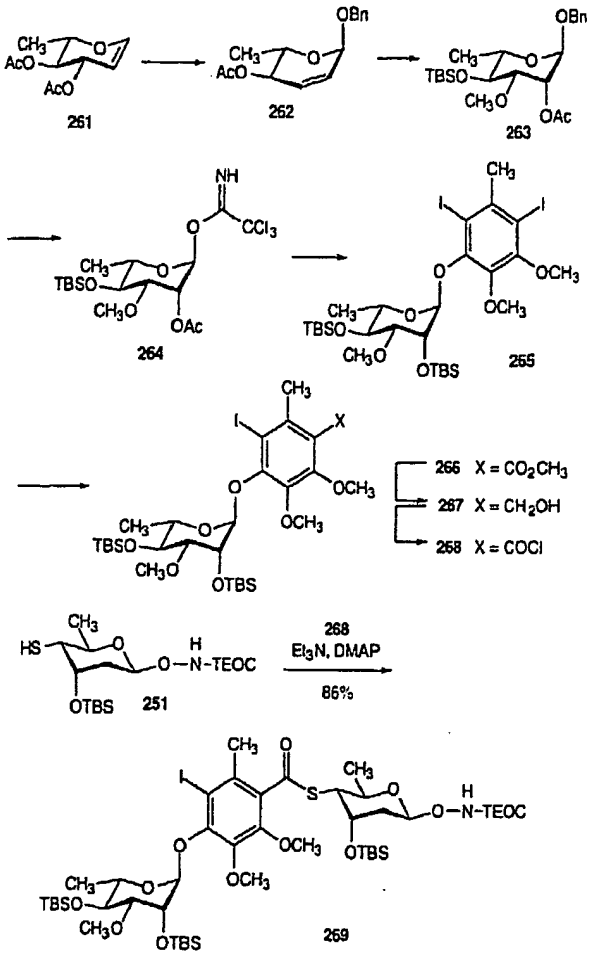


Scheme 60. Synthesis of the esperamicin carbohydrate domain (part 3).

the PMB groups. At this point, compound **260** was in hand. The *N*-TEOC group at the hydroxylamine linkage prevented the ensemble from rearranging to an azafuranose. Indeed, when construct **260** was exposed to the action of TBAF all silyl-based protecting groups were cleaved. However, compound **232**, which would correspond to the fully deprotected core trisaccharide domain of esperamicin, was not isolated or observed. Rather, the isolated product was the azafuranose **233a** and was best characterized as the methyl glycoside **233b**. This compound proved to be identical with a sample that had been derived from esperamicin by the chemists at Bristol Myers (vide supra).

At this stage, it was clear that for the core trisaccharide to maintain viability either the spacer hydroxylamine or the anomeric center at the reducing end must be protected. Since preparation of a glycosyl donor to serve as a calicheamicin-directed construct would probably require the intermediacy of a construct with a free reducing end, it would be necessary to maintain protection of the NH group of the hydroxylamine spacer center during this fashioning stage.

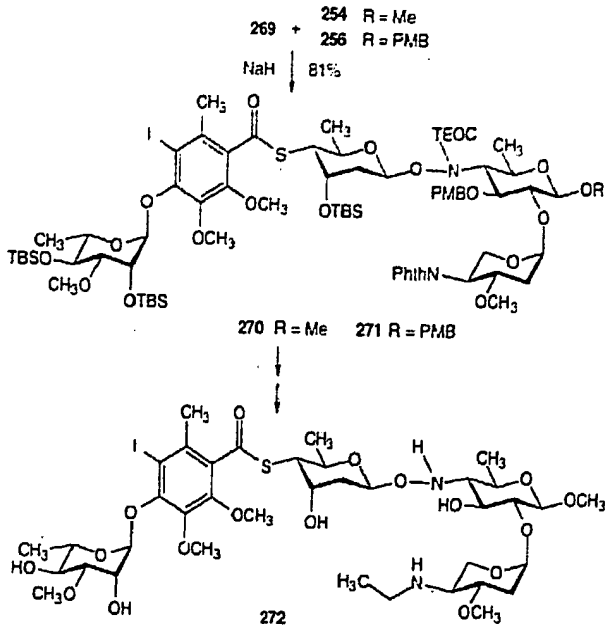
Our attention was next directed to the aryltetrasaccharide domain of calicheamicin. Of course, the synthesis of the ABC sector of **230** would be closely patterned after the program followed in the esperamicin series. Thus, we focused on the CD sector of the domain (Scheme 61). We started with glycal **261**,



Scheme 61. Synthesis of the calicheamicin carbohydate domain (part 1).

which was available from L-rhamnal. Ferrier rearrangement with benzyl alcohol afforded **262**, which was converted by osmylation, monomethylation (of the derived stannylene), and silylation to provide **263**. After debenzylation, the trichloroacetimidate donor function was introduced, affording **264**. Schmidt coupling of **264** to 2,4-diiodo-5,6-dimethoxy-3-methylphenol afforded **265**. Regioselective palladium-mediated carbonylation furnished **266**, which upon reduction (to **267**), followed by oxidation and formation of the acid chloride, provided **268**.[119] The previously described C-ring thiol-containing glycal **251** underwent acylation with **268** to afford aryl disaccharide **269**.

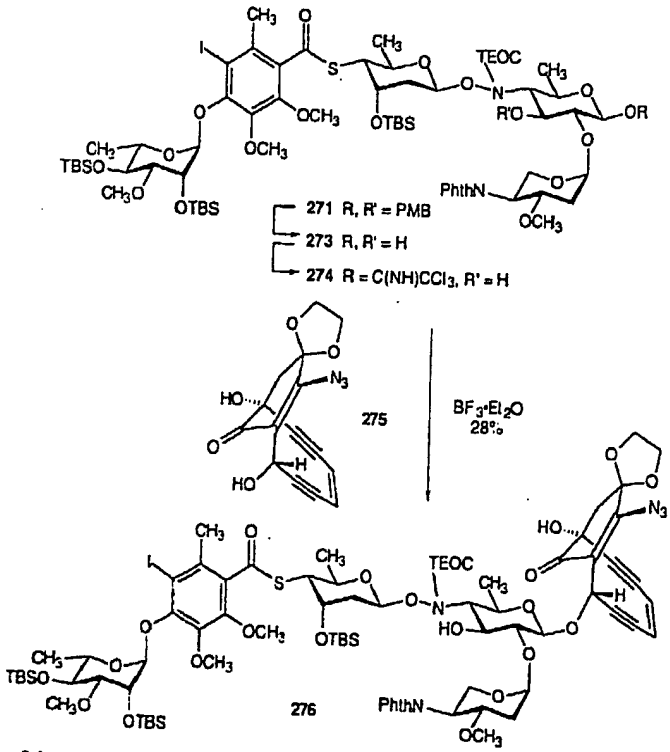
Kahne coupling[118] of the anion of **269** with the triflate **254** produced the aryl tetrasaccharide **270** (Scheme 62). Removal of the phthalamide group was followed by reductive ethylation and cleavage of all silyl-based blocking groups and the PMB ether to provide **272**. A variety of independent investigations by our group[120–122] and by Nicolaou et al.,[123,124] who had earlier synthesized the carbohydrate domain,[125] revealed that



Scheme 62. Synthesis of the calicheamicin carbohydrate domain (part 2).

the recognition attribute of calicheamicin is, in fact, vested in the aryltetrasaccharide domain embodied in 272.

Our next goal was to demonstrate that a competent glycosyl donor could be fashioned from 272 and, above all, delivered to a reasonably advanced version of the aglycone domain. For this purpose, 269 and 256 were coupled to provide aryl tetrasaccharide 271 (Scheme 62). Deprotection of 271 with 2,3-dichloro-5,6-dicyanobenzoquinone (DDQ) afforded 273, which was converted to the trichloroacetimidate donor 274. Among the aglycone acceptors screened was 275. Schmidt coupling of 274 and 275 indeed gave rise to a 3:1 mixture of 276 and the corresponding α-glycoside, which were readily separable (Scheme 63). This was the first such demonstrated glycoside

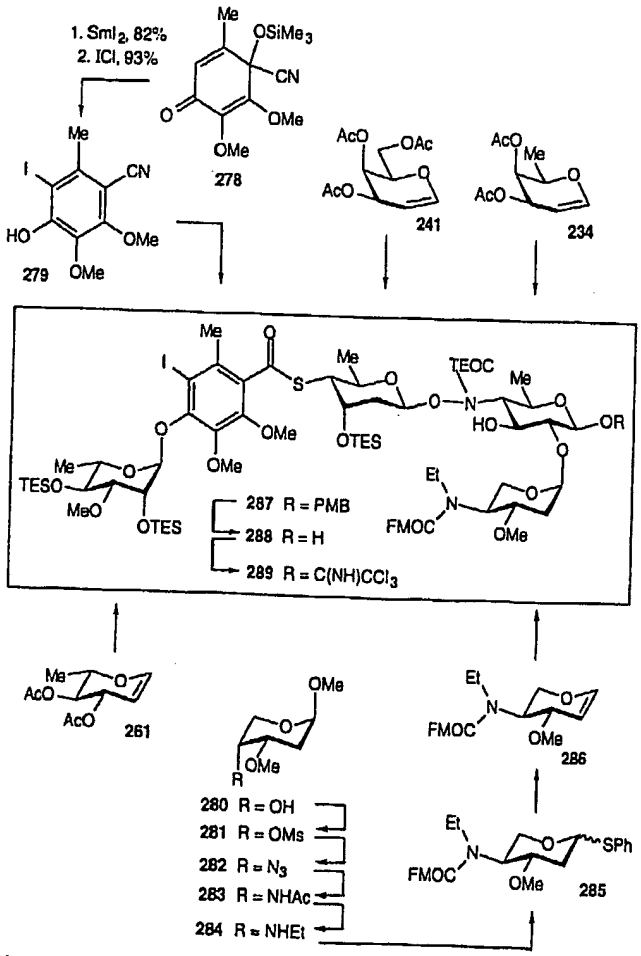


Scheme 63. Coupling of the carbohydrate sector to an enediyne core.

coupling of two fully synthetic domains in the calicheamicin series.[126]

While the result was certainly pleasing, it soon became clear that compound 276 was insufficiently developed for advancement to fully synthetic calicheamicin. During this era of the program, several valuable insights were garnered. A key finding was that hydrazine-induced cleavage of the phthalimide group was not possible in the presence of the cyclic enediyne functionality. Under various conditions, destruction of the enediyne network (albeit in an uncharacterizable way) occurred far more rapidly than liberation of the amine from the phthalimide. Similarly to be taken to heart, was the finding that cleavage of the axial TBS protecting groups with TBAF was a problematic step. When the final deprotection was conducted on various constructs containing these protecting groups, the rhamnose D ring was seriously damaged or lost. This loss could be minimized at low temperatures (−10 °C), but under these conditions deprotection of the axial TBS groups took roughly seven days. Based on the results of experiments in which the "survival times" of these functional groups were determined, it seemed unlikely that an enediyne moiety could withstand the action of these conditions for the period of time required for freeing of the alcohol from its TBS derivative.

In response to these problems, and taking cognizance of the first total synthesis of calicheamicin by Nicolaou and colleagues,[111] we changed our pattern of protecting groups to that shown in Scheme 64. In preparing for the final push toward calicheamicin γ_1^I, several processes were improved. The route to

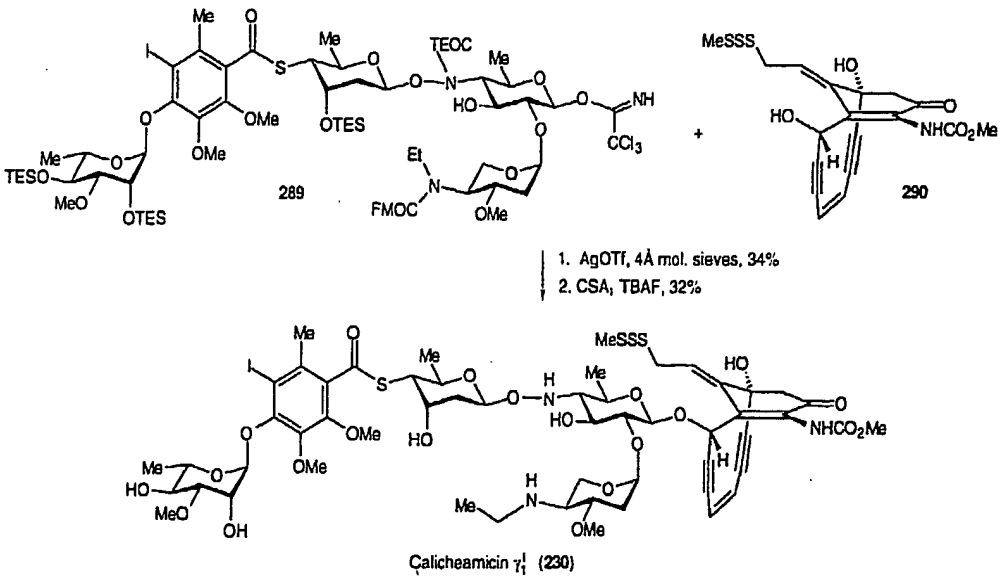


Scheme 64. Improved synthesis of the calicheamicin carbohydrate domain. FMOC = 9-fluorenylmethyloxycarbonyl.

the aromatic sector was totally revamped and streamlined. A key finding, per se unrelated to carbohydrate chemistry, was registered by Steven Olson. He found that mono-TMS cyanohydrin derivatives of quinones (e.g. **278**) are converted to *p*-hydroxybenzonitriles (e.g. **279**) through the action of samarium(II) iodide.[127] D-fucal (**234**), D-galactal triacetate (**241**), and L-rhamnal diacetate (**261**) were the building blocks for the A, C, and D rings, essentially as before, with the exception that triethylsilyl groups were employed instead of TBS functions as the protecting groups for the hydroxyl groups.

The starting material for the 9-fluorenylmethyloxycarbonyl (FMOC)-containing glycal was mesylate **281**, derived from differentiated methylglycoside **280**. Serge Boyer found that azide displacement upon **281** afforded **282** and, in seriatum, the acetamide **283** and then the *N*-ethyl derivative **284** were fashioned. Thiophenol displacement at the anomeric center provided **285**, which upon oxidation and thermolysis afforded **286**. Glycal assembly in analogy to our first calicheamicin synthesis used **237** for the iodoglycosylation with **286**, en route to the AB section. Continuation of the sequence led to tetrasaccharide **287**, thence to **288**, and finally to **289**.

At this stage Steven Hitchcock was able to investigate a most exciting possibility. Glycosylation could be conducted with **289** as the projected donor and **290** as the acceptor (Scheme 65). The feasibility of employing such a structurally advanced acceptor arose from the use of donor **289**. Since no oxidations



Scheme 65. The ultimately convergent synthesis of calicheamicin.

or reductions would be necessary to bring the projected product to the state required in the drug, one would hope to use the otherwise vulnerable acceptor **290**, in which the allylic trisulfide functionality is already present. Fortunately, reaction under particularly mild conditions (AgOTf, 4Å molecular sieves)[128] allowed for glycosylation. All blocking groups were discharged in two steps, and calicheamicin γ_1^I (**230**) was in hand. Thus, an ultimately convergent synthesis had been accomplished.

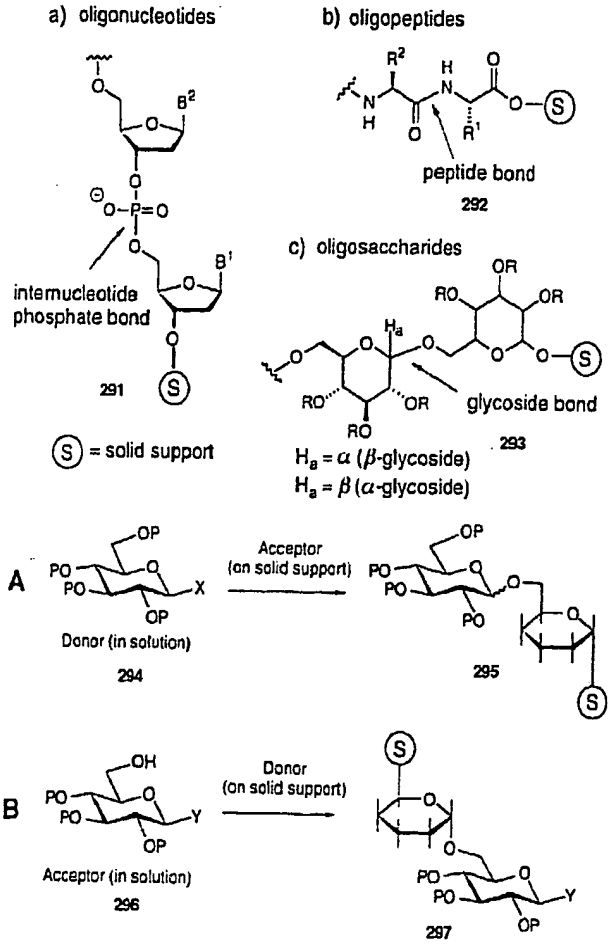
10. Solid-Phase Oligosaccharide Synthesis

Progress in the synthesis of oligosaccharides and glycoconjugates by the solution-based methodology described in the previous sections was certainly reassuring. Yet because of the importance of this field, these advances prodded us to seek still greater levels of simplicity and efficiency. It was instructive to think about this problem in the broader context of biooligomer synthesis, thus inviting analogies between oligosaccharide synthesis and the synthesis of oligonucleotides and peptides. Of course, impressive advances had been registered in the solution-phase synthesis of these latter biooligomers. However, it is clear that the major upsurge in their synthesis arose only after solution-based coupling methods were adapted to the solid phase. While polymer-supported synthesis of oligopeptides[129] and oligonucleotides[130] is not a panacea, it has certainly been of enormous benefit in improving yields, simplifying procedures, and obviating the need for purification at each stage. Obviously, in solid-phase methodology the potential advantage of such purification after each elongation is substantially forfeited. Hence the chemistry of the various reiterations must be sufficiently efficient that a single purification at the final stage affords product of the required homogeneity.

In polypeptide and oligonucleotide synthesis, it is the high yields in the individual coupling steps that seem to render the final-stage purification strategy viable. Excellent yields arise from the inherent quality of the coupling steps and are further "amplified" by the capacity to employ excess solution-based coupling partner, which is removed from the solid phase by filtration.

By contrast, solid-phase synthesis of oligosaccharides is much less developed.[131] This assessment is in no sense intended as a critique of the practitioners of the field; rather it reflects the fact that the problem of oligosaccharide synthesis is intrinsically far more complicated than the corresponding problem in oligonucleotide and oligopeptide synthesis (Scheme 66).

Consider the synthesis of oligonucleotides, particularly oligo-2-deoxynucleotides (Scheme 66). Assuming the availability of the individual nucleosides, each elongation involves the fashioning of an internucleotide phosphate bond. For this purpose it is necessary to distinguish between the 5'- and 3'-hydroxyl groups and to discover a high-yielding coupling step. The fashioning of the internucleotide bond does not create a new chiral center. Similarly, in fashioning a peptide the α-amino and α-carboxyl groups must be distinguished from any such or related functionality (for example thiol and hydroxyl groups) present on various side chains. An efficient coupling is necessary for amide bond forma-



Scheme 66. Solid-phase synthesis of biopolymers. B^1, B^2 = nucleobases.

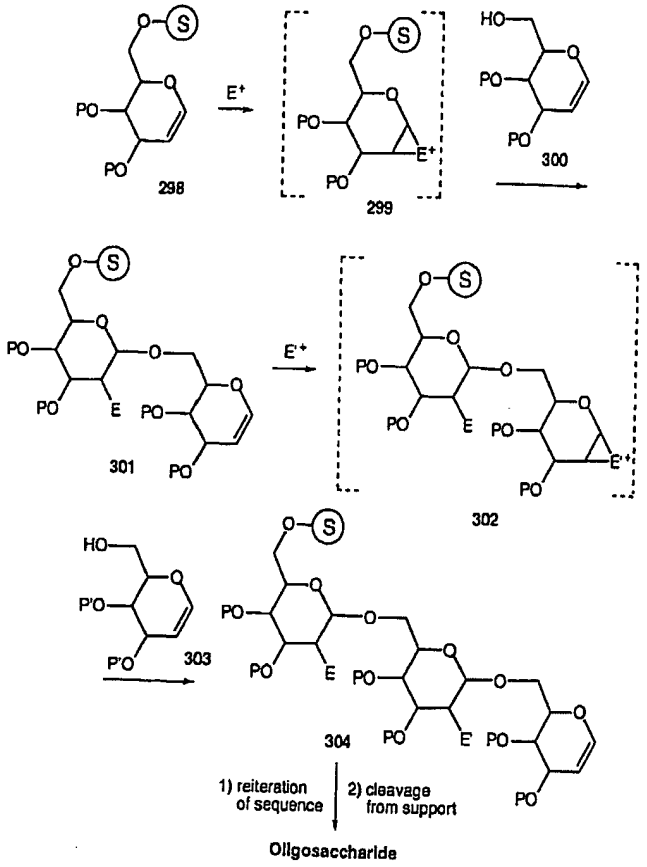
tion. Once again, no new chirality arises in the elongation of the oligopeptide. The two strategies for elongation of these biopolymers on solid supports are implied in structures 291 and 292 in Scheme 66. Clearly, in contemplating the synthesis of an oligosaccharide on solid support (cf. 293) the complexity level rises markedly.

Thus, in fashioning the repeating units from an aldohexose, one must distinguish the anomeric region of one of the components to serve as the donor region (see 294). In the case of combining hexose units, one must also differentiate one of five rather closely related hydroxyls to serve as the glycosyl acceptor center (see 296). Most demanding is the need to control the configuration at each newly emerging glycosidic bond. Unlike the synthesis of the other biooligomers, the linkage of saccharide monomers through formation of a glycoside bond has serious stereochemical consequences. Given the enormously more complicated nature of the problem of oligosaccharide synthesis on a solid support, the dramatic progress that has been registered is indeed amazing and speaks eloquently for the creativity and tenacity of its founding practitioners.

In contemplating the syntheses of oligosaccharides on a solid support, two overall strategies can be entertained. In one instance (Case A) a glycosyl acceptor is mounted to a support, and solution-based donor (294) as well as promoter are administered for the coupling step leading to glycoside 295. To reiterate the process, a new acceptor must be fashioned on the support. This would generally involve cleavage of a specific protecting group (P) to generate a new acceptor center in a defined position.

In the alternative strategy (Case B) the polymer-bound glycosyl donor reacts with the solution-based acceptor (296). For reiteration, the donor functionality must be unveiled from the terminal anomeric functionality (Y) on the support-bound structure (297). Also, positionally defined glycosyl acceptors must be synthesized for each iteration such that acceptor character is manifested at a particular hydroxyl center and donor character can be fashioned at the anomeric center of the product. The incremental complexities of oligosaccharide synthesis on a solid support relative to that associated with the other classes of biooligomers is virtually palpable upon analysis of the problem.

It was in dealing with the problem of solid-phase carbohydrate synthesis that we felt that glycal-based constructions might prove to be particularly valuable.[132,133] The guiding paradigm was that shown in Scheme 67. Polymer-bound glycal

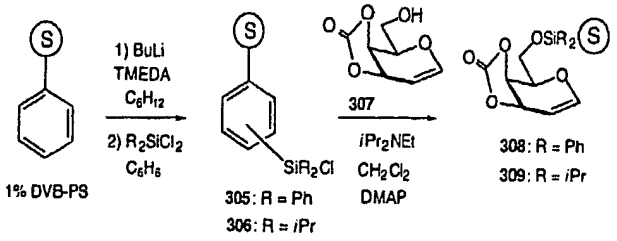


Scheme 67. Solid-phase carbohydrate synthesis employing glycals. P, P' = protecting groups; E^+, E'^+ = electrophiles.

298 would be synthesized by attaching the requisite glycal to a suitable solid support. The system would be activated by unspecified electrophile E^+ to furnish polymer-bound donor 299. In principle 299 can be a substoichiometric intermediate (cf. iodoglycosylation) or a characterizable chemical entity (cf. 1,2-epoxide). Coupling of 299 with solution-based glycal acceptor 300 would give rise to the elongated polymer-bound glycal 301. Reiteration of the process generates 304 via new polymer-bound donor 302 and solution-based acceptor 303 (which may or may not be identical to 300).

Several decisions, starting with the choice of polymer, were necessary to study the implementability of the scheme. We elect-

ed a silicon-based attachment of the growing carbohydrate domain to the solid support and turned to commercially available polystyrene (cross-linked with 1% divinylbenzene). Fortunately, we were able to take good advantage of the findings of Chan[134] registered in a totally different context. Thus, metalation of the polymer leads to formation of the aryllithium species. When exchange is followed by silylation with a difunctional silane of the type R_2SiCl_2, a silyl chloride functionalized resin is obtained (Scheme 68).

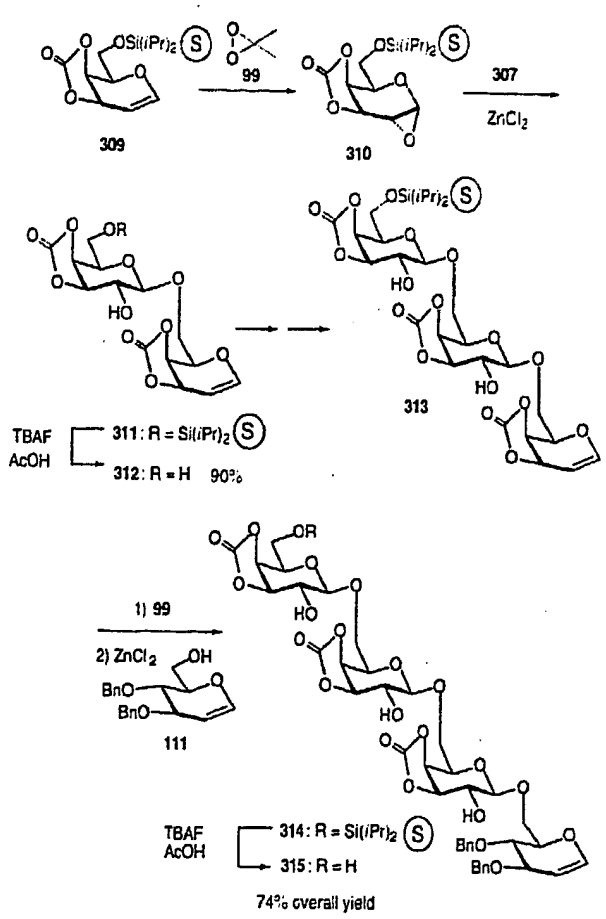


Scheme 68. Preparation of a polymer-linked glycal. TMEDA = tetramethylethylenediamine. 1% DVB-PS = polystyrene cross-linked with 1% divinylbenzene.

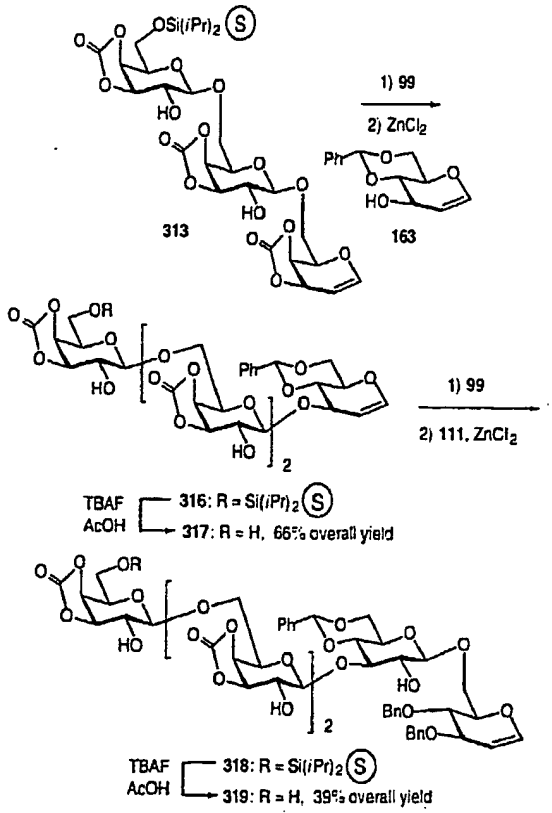
In our initial explorations[132] we silylated with diphenyldichlorosilane and attached the first glycal (307) through a conventional silylether forming reaction to provide 308. Subsequent studies revealed that the spacer in the products arising from elongation of 308 lacked the needed hydrolytic stability. Accordingly, we turned to the use of diisopropyldichlorosilane as the silylating agent, which led us to 309 as our support-bound donor of choice. We determined the loading of carbohydrate to be in excess of 0.9 mmol per gram of 307. The activation method we developed at first was that of glycal epoxidation using 2,2-dimethyldioxirane as the oxidant. Of course, from this point on the support-bound compounds (e.g. 310) were generally not fully characterizable (Scheme 69). Reaction of 310 with glycal acceptor 307 mediated by zinc chloride afforded 311. We could establish the actual presence of 311 by treatment with tetra-*n*-butylammonium fluoride (TBAF), which provided 312 in roughly 90% yield. Reiteration of the sequence, twice more, using acceptors 307 and 111 in sequence followed by removal from the polymer with TBAF led to tetrasaccharide 315 in 74% overall yield (approximately 90% average yield per coupling step). The reader will recognize that in these early forays we relied heavily on galactose epoxide donors of the type 310. These tend to offer high margins of β-product formation (see Scheme 37). That galactosylation is also an important capability in fashioning biologically important oligosaccharides had not escaped our attention.

Several features of the method should be emphasized. First, the polymer-bound donors in which the C3 and C-4 hydroxyl groups are engaged as a cyclic carbonate are, in fact, highly stereoselective galactosylating agents. Single purification at the tetrasaccharide stage was a straightforward matter. Another feature was the "self-policing" nature of failed couplings. While the average coupling yields are only about 90%, the uncoupled epoxide is apparently destroyed by hydrolysis. Thus, we do not encounter entities with deletions in the interior of the chain.

Glycosyl acceptors with secondary hydroxyl groups are also accommodated by this method (Scheme 70). Compound 313,



Scheme 69. Solid-phase synthesis of 1,6-linked polysaccharide residues.



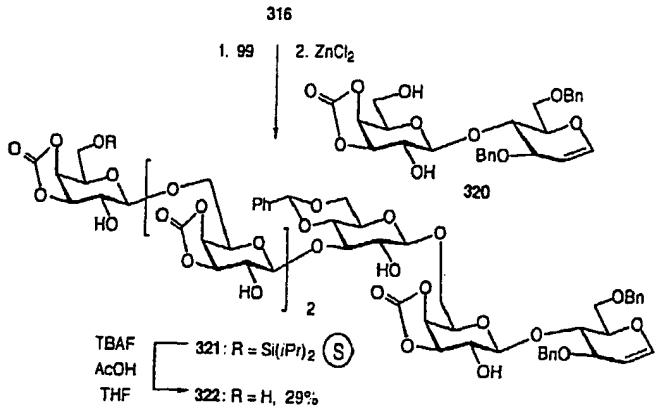
Scheme 70. Synthesis of a pentasaccharide on a solid support.

following epoxidation with 99, reacted with D-glucal derivative 163 to give 316. Tetrasaccharide 317 was retrieved from the support by the action of TBAF in a 66% overall yield based on 309. Assuming 90% yield per coupling in the synthesis of 313,

glycosidation of 163 had occurred in roughly 80% yield. An additional example demonstrating the use of glycals with secondary hydroxyl groups is the coupling of solution-based 3,6-dibenzylglucal with 310 to give the corresponding 1,4-linked disaccharide product.

Compound 316 was oxidized with 99 to give a polymer-bound glucosyl donor. This epoxide reacted with $ZnCl_2$ and a solution of 111 in THF to provide 318, which was cleaved from the support to give pentasaccharide 319 in 39% overall yield from 309. This glycosylation, which had been achieved in approximately 60% yield based upon 316, also occurred with a high degree of stereoselectivity. However, in this particular case, a minor component, believed to be the α-glycoside, was detected in the ^1H NMR spectrum. The ratio of the desired all-β-glycoside product to this unknown component was in excess of 10:1. This apparent erosion from strict β selectivity using this glucal-derived donor can perhaps be controlled by appropriate modification of the as yet unoptimized conditions.

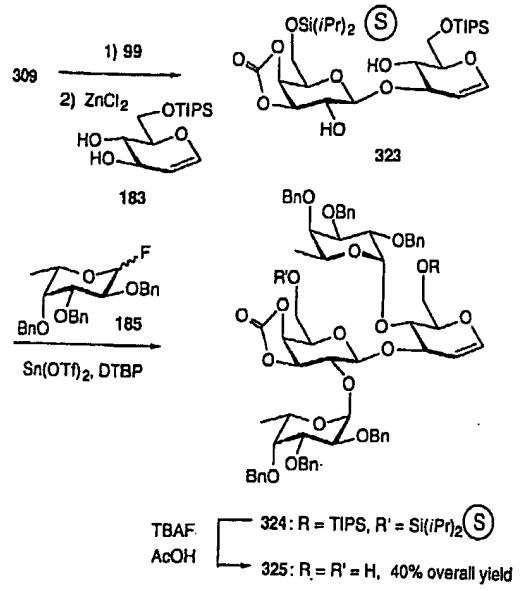
The scheme can be rendered more convergent through recourse to disaccharide and higher oligomer acceptors (Scheme 71). Thus, epoxidation of polymer-bound tetrasaccharide glycal 316 followed by zinc chloride mediated coupling with disaccharide acceptor glycal 320 and retrieval from the support (TBAF) afforded 322 in a 58% overall yield (29% overall from 309, 45% yield from 316).



Scheme 71. Synthesis of a hexasaccharide on a solid support.

It should be noted that this last coupling involves the regioselective glycosylation of a diol acceptor. This element of "site direction" in the use of polymer-bound donors with solution-based acceptors can be a significant advantage since protecting group manipulations can be minimized.

The solid-phase method is also applicable to the synthesis of branched structures through the logic of glycal assembly (see Schemes 41 and 42). We demonstrated the principle with support-bound glycal 309, which was epoxidized and coupled to acceptor 183 to afford disaccharide 323 (Scheme 72). The latter served as a polymer-bound glycosyl acceptor in a reaction with fluorosugar 185 mediated by stannous triflate. Tetrasaccharide 324 was retrieved from the polymer with TBAF to afford 325, a glycal precursor of Lewis b. Thus, it was demonstrated that branching at the C2 hydroxyl could be achieved in a growing chain by exploiting the hydroxyl group unveiled in the epoxide donor based glycosylation.



Scheme 72. Synthesis of branched sugars on a solid support. DTBP = di-*tert*-butylperoxide.

We note that at this stage direct sulfonamidoglycosylation of solution-phase carbohydrate acceptors with polymer-bound donors (cf. Scheme 22) was not possible. While halosulfonamide adducts of glycals can be formed on supports, the glycosidation failed. Thus, we were unable to make our way from polymer-bound 324 to the full Lewis b determinant. Instead, we had to revert to solution-phase methods (see 325) and complete the Lewis b synthesis by the previously discussed procedure. More recently we have developed a two-stage protocol for conducting sulfonamidoglycosylation on support-bound substrates (see Scheme 81).

Another of our goals was to build upon the capabilities attained in glycal assembly to synthesize glycopeptides. Our first objective was to reach asparagine-linked glycopeptides. Brilliant advances in glycopeptide synthesis have been achieved by many researchers,[135] including notably, Paulsen, Kunz,[135e] Meldal, and Lansbury.[135e] The strategy we hoped to implement would be radically different and maximally convergent.

It was envisioned that a terminal glycal of a synthetic oligosaccharide domain would be subjected to iodosulfonamidation. As demonstrated earlier in a simpler model (see 90 → 91, Scheme 25), treatment of such an intermediate with azide results in formation of β-anomeric azide with suprafacial movement of the α-sulfonamide from C1 to C2. Reduction of the azide and acylation of the resultant anomerically pure β-aminosugar provided a protected glycopeptide (93, Scheme 25). Fortunately, this capability was tranferable to the solid phase (vide infra).

This was to serve as the cornerstone of our strategy. First, however, a cautionary note is appropriate. While the synthesis of glycopeptide ensembles is a complex undertaking, the most difficult part of the enterprise may actually be the final maneuvers required to produce the fully deprotected entity. Thoughtful planning and corresponding care must be exercised since the *N*-asparagine-linked glycopeptide can be a rather vulnerable construct.

Jacques Roberge took up the problem in great detail and was subsequently joined by Xenia Beebe. In this presentation we spare the reader the description of the many setbacks encoun-

tered in implementing the seemingly straightforward design with provision for full deprotection. We will first present the solution synthesis of glycopeptides by this method[136] and then demonstrate its compatibility with synthesis on solid supports.

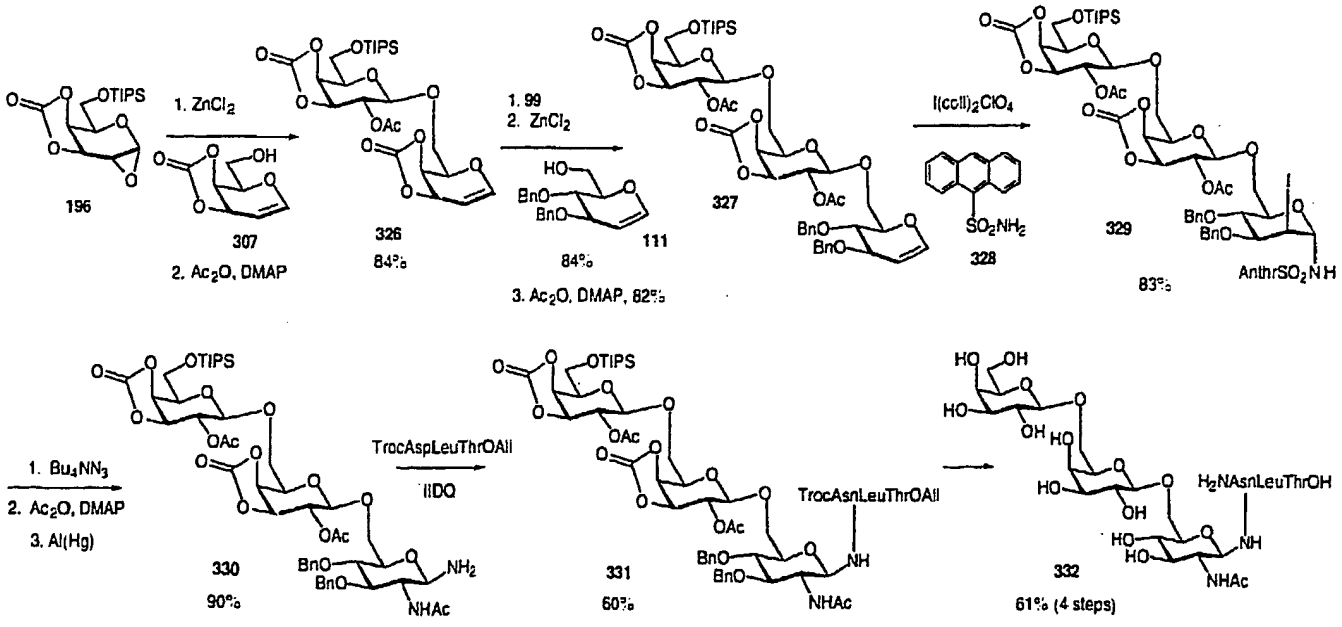
In synthesizing the carbohydrate domain of the glycopeptide we focused on a target structure amenable to the most straightforward methodology we had developed. For this purpose, we relied on the galactal epoxide **196**, which reacted with **307** to give, after acetylation, disaccharide **326** (Scheme 73). Epoxidation of **326** followed by reaction with acceptor **111** furnished, after acetylation, the trisaccharide glycal **327**. This set the stage for functionalization of the glycal linkage with a view to glycopeptide formation. In pursuit thereof, we eventually settled on the use of 9-anthracenylsulfonamide (**328**) in the iodosulfonamidation reaction. We have demonstrated that this protecting group can be readily removed under a variety of mild reducing conditions.

Treatment of **327** with **328** and di-*sym*-collidine iodonium perchlorate gave rise to **329**. Reaction of the latter with tetra-*n*-butylammonium azide triggered the expected relocation of the sulfonamide. The resulting azidotrisaccharide was then acetylated. Azide reduction and reductive cleavage of the sulfonamide was accomplished with aluminum amalgam to afford **330**. Acylation of the β-amine was conducted with the ω-carboxyl group of the tripeptide TrocAspLeuThrOAll in the presence of IIDQ, giving rise to **331**. Deprotection was accomplished by desilylation, removal of the allyl group, reductive removal of the Troc group, and hydrogenolysis of the benzyl groups. Lastly, the two cyclic carbonate linkages were cleaved by KCN/ethanol and the free N-linked glycopeptide **332** was in hand. We view this synthesis of the trisaccharide tripeptide ensemble as an important plateau in aspiring to the synthesis of glycopeptides.

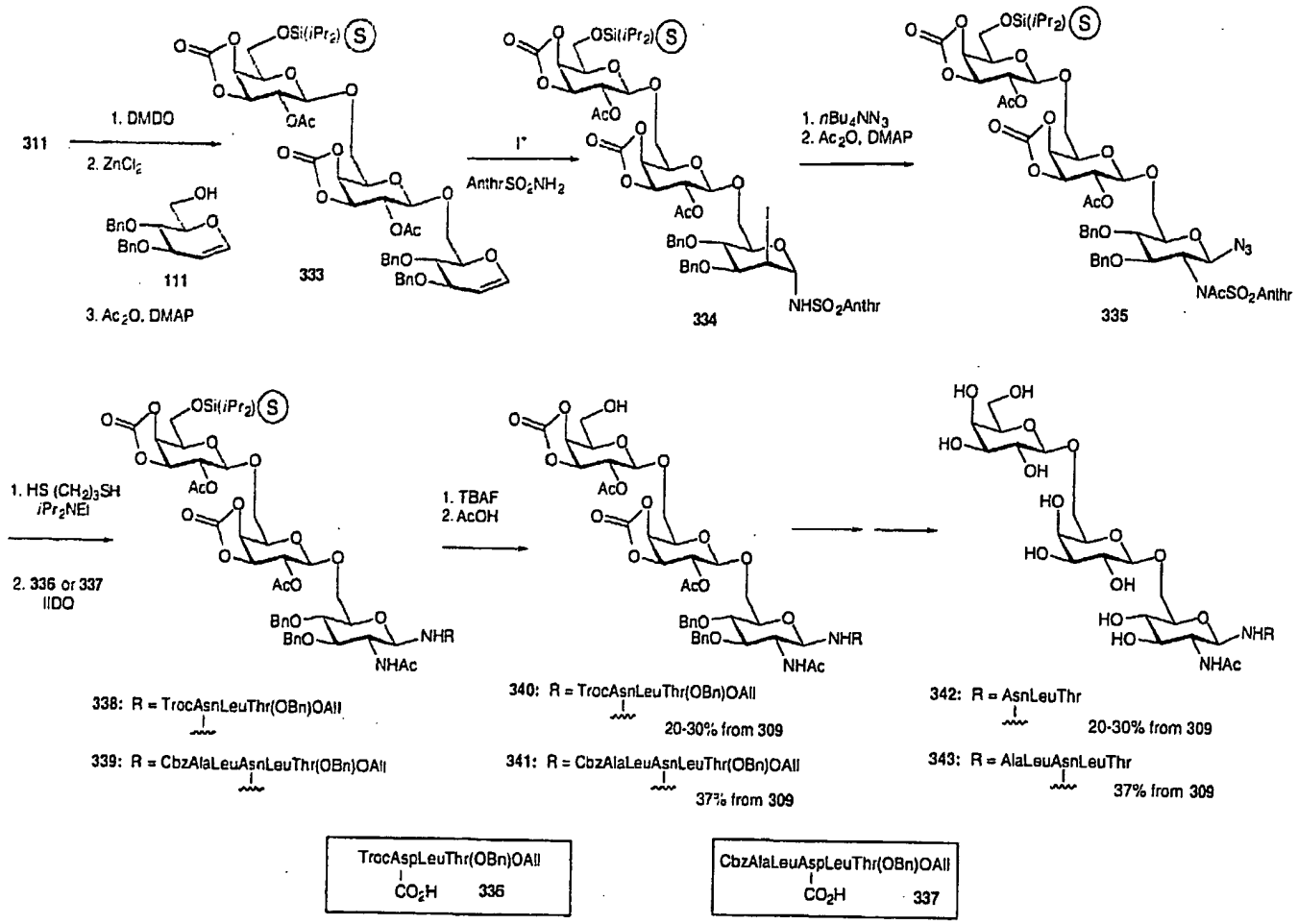
Having demonstrated the feasibility of the synthesis in solution, we next turned to conducting it on the solid support (Scheme 74).[137] Polymer-supported disaccharide **311** was extended by epoxidation, reaction with **111**, and acetylation to provide trisaccharide **333**. After treatment of **333** with anthracenesulfonamide and I(*sym*-coll)$_2$ClO$_4$, the clean formation of **334** was inferred from subsequent steps. Reaction of this material with tetra-*n*-butylammonium azide followed by acetylation provided the anomeric azide **335**. This success is in contrast to our inability to achieve polymer-based sulfonamidoglycosylation of carbohydrate acceptors (see Scheme 71).

The principal advantage in using the anthracenesulfonamide linkage is that it can be cleaved by a variety of mild methods. For instance, we developed the use of thiophenol or 1,3-propanedithiol and Hünig's base for the removal of the anthracenesulfonyl group. These protocols are compatible with synthesis on solid supports. Also, anthracenesulfonamide itself is more soluble than benzenesulfonamide in THF, which is a good swelling solvent for the polymer. Thus, the use of the anthracene-based agent results in a more efficient and complete iodosulfonamidation reaction. Treatment of **335** with 1,3-propanedithiol and *i*Pr$_2$NEt effected both the reduction of the azide and removal of the sulfonamide. The resulting amine was coupled with tripeptide **336** and alternatively with pentapeptide **337** in the presence of IIDQ to afford the protected glycopeptides **338** and **339**, respectively. Removal from the solid support with HF·pyridine provided the glycopeptides **340** and **341** in overall yields of 30 and 37%, respectively. For the synthesis of **341** this constitutes an average yield of approximately 90% per step over the ten steps from polymer-bound glycal **309**. Chromatography on a short column of reversed-phase silica gel (C-18) was sufficient to obtain **340** and **341** in pure form. This ready purification can be attributed to the previously described "self-policing" feature of the solid-phase glycal assembly method (see Scheme 70) and illustrates the efficiency in the conversion of the terminal glycal to the terminal glucosylamine.

The remaining protecting groups in both **340** and **341** were cleaved under standard conditions to provide the completely deblocked glycopeptides, **342** and **343**, in yields of 61 and 48% (from **340** and **341**), respectively. Structural characterization of the glycopeptides by NMR spectroscopy confirmed the β-con-



Scheme 73. Synthesis of N-linked glycopeptides. All = allyl. Anthr = anthracenyl. Troc = trichloroethoxycarbonyl. IIDQ = 2-isobutoxy-1-isobutoxycarbonyl-1,2-dihydroquinoline.

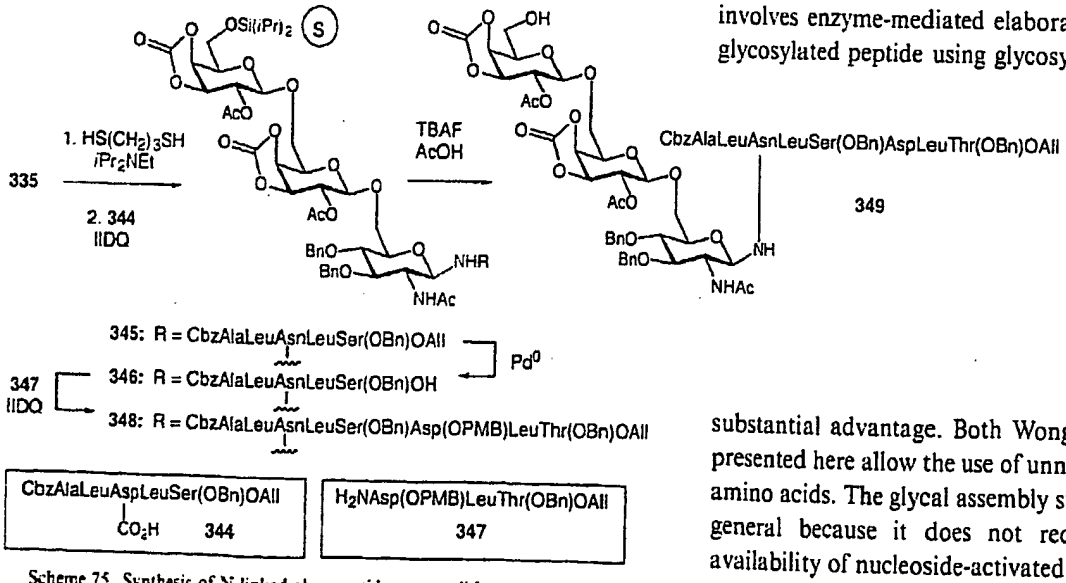


Scheme 74. Synthesis of N-linked glycopeptides on a solid support. Cbz = benzyloxycarbonyl.

figuration of all the anomeric linkages. The structures were further corroborated by mass spectroscopy.

The presence of orthogonal protecting groups on the C- and N-termini of the peptide provides the opportunity to extend the peptide chain while the ensemble is bound to the solid support. Alternatively, after removal from the support, the liberated peptide terminus may provide a functionality for linking to a carrier molecule to generate other glycoconjugates. Scheme 75 shows how the peptide portion of the glycopeptide was extended while still bound to the polymer support. The solid phase bound trisaccharide pentapeptide **345** was assembled as previously described from **335** by employing pentasaccharide **344** in the coupling reaction. The C-terminus of **345** was deprotected to give acid **346**, which was then coupled to tripeptide **347** with a free N-terminus to give glycopeptide **348**. Retrieval from the solid support afforded the trisaccharide–octapeptide **349** in an 18% overall yield from polymer-bound galactal carbonate.

In contemplating the future of glycopeptide synthesis, one should take particular note of the method of Wong,[138] which involves enzyme-mediated elaboration of a solid phase bound glycosylated peptide using glycosyl transferases to append unprotected nucleoside phosphate activated monosaccharides in the elongation phase. The conjugate is retrieved from the solid support by protease action. Protecting group manipulations are not necessary in enzymatically mediated chemistry, which can be a substantial advantage. Both Wong's method and the method presented here allow the use of unnatural amino acids and non-amino acids. The glycal assembly strategy is in principle totally general because it does not require transferases and the availability of nucleoside-activated hexoses. It can also accommodate the inclusion of unnatural (artificial) sugars in the



Scheme 75. Synthesis of N-linked glycopeptides on a solid support.

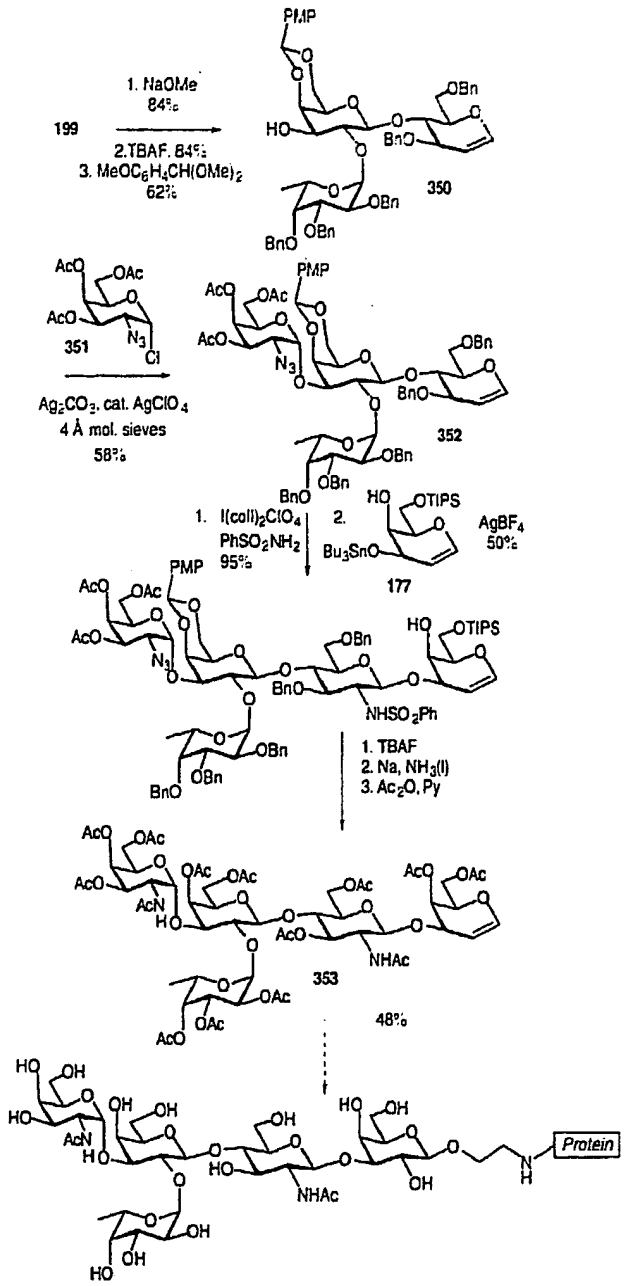
construction. Such building blocks are available from the Lewis acid catalyzed diene–aldehyde cyclocondensation reaction (Scheme 2). All workable approaches, whether purely chemical or chemo-enzymatic, are complementary for reaching the common goal of carefully designed, fully synthetic glycopeptides.

11. Studies in Progress

Thus far we have confined ourselves to a review of programs that have been completed and published. It is well to bring the reader up to date on current efforts in our laboratory. This update will help to underscore the sorts of chemical and biological problems we are addressing through the strategy of glycal assembly.

11.1. Synthesis of Blood Group A (Type 2) Determinant

We have been continuing our work in the total synthesis of human blood group determinants using glycal assembly. An important recent target has been the blood group A (type 2) determinant.[7] While we have not yet reached this goal, in the sense of producing a fully deprotected, suitably conjugated blood group A substructure, the substantial progress realized[139] suggests that this will be achieved shortly. The synthesis started with previously described compound 199, which was converted to glycal 350 such that the C3'-hydroxyl of the galactose moiety could subsequently function as the glycosyl acceptor site (Scheme 76). Fortunately, this coupling could be accomplished by using the anomerically pure C2-azido chloride 351, as pioneered by Paulsen.[140] The resulting 352 has now been converted by our standard protocols to the pentacyclic glycal 353. The latter awaits suitable conjugation to provide the synthetic human blood group A determinant and immunological investigation.



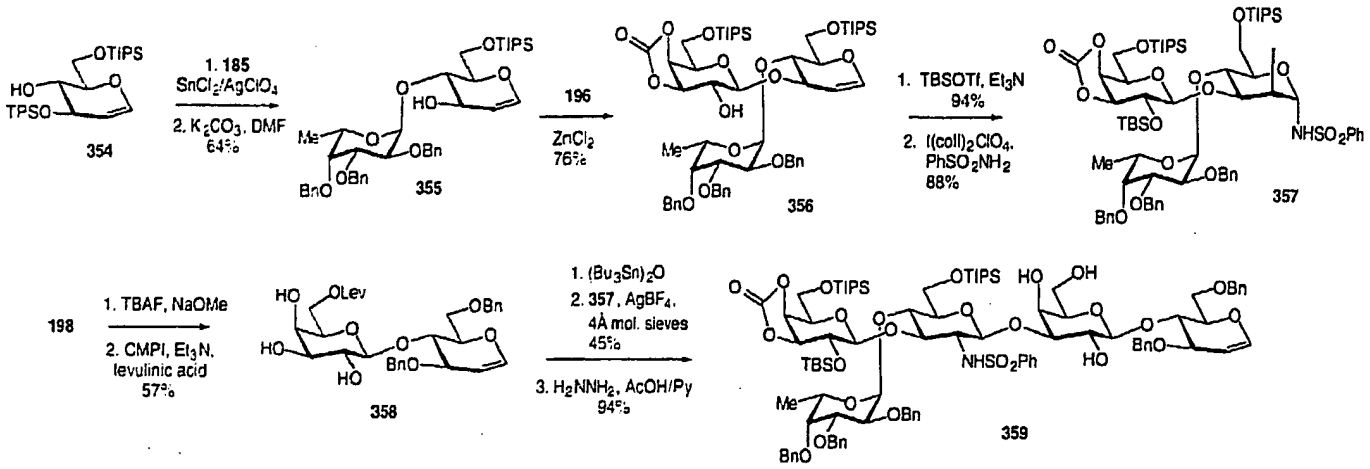
Scheme 76. Synthesis of a glycal of the blood group A (type 2) determinant.

11.2. An Approach to the N3 Antigen

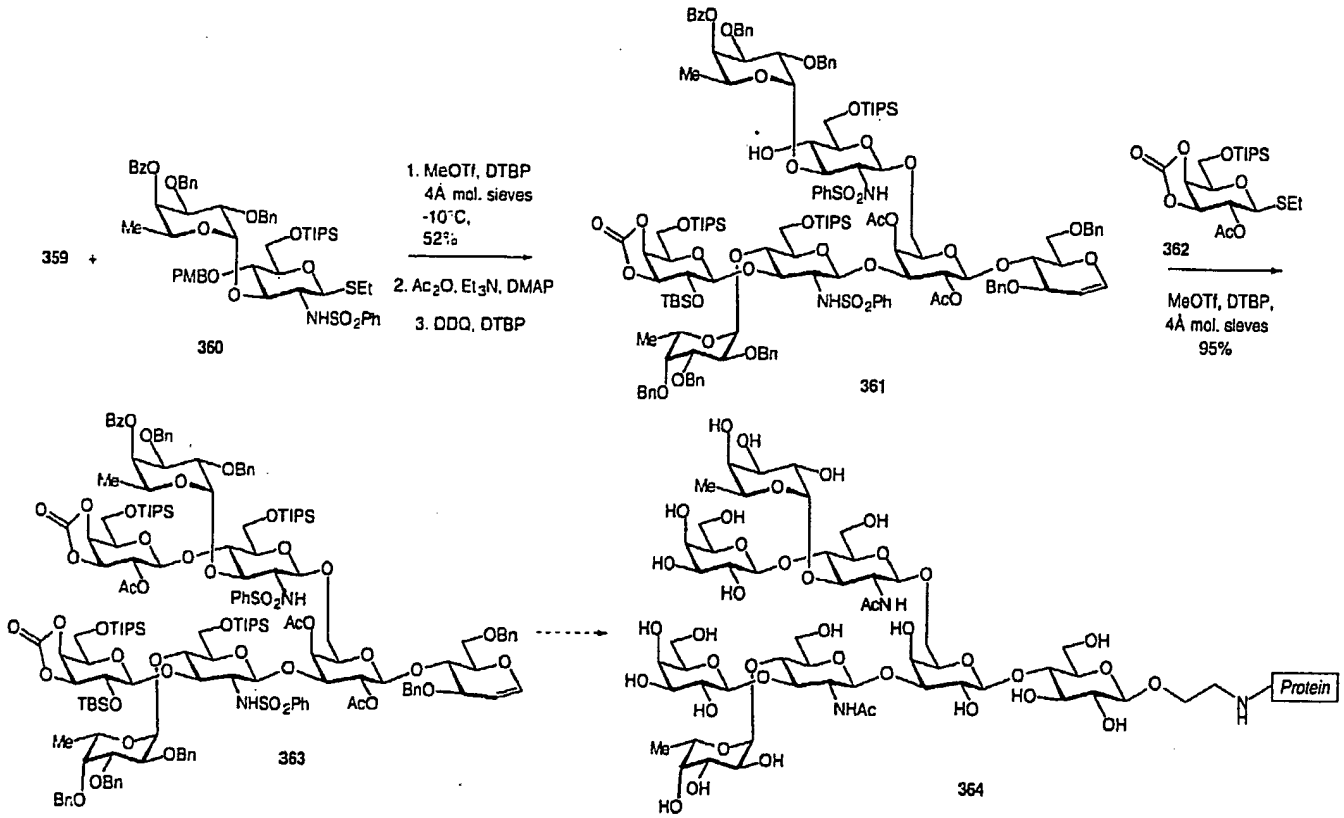
Our synthesis of the N3 antigen is now nearing completion. We, as well as others,[141] have high hopes that a suitably conjugated version of this antigen can be used to detect even minuscule amounts of the N3 antibody, which appears to be specifically produced in response to gastrointestinal cancer.

The realization of this synthetic goal by glycal assembly methodology turned out to be more complicated than might have been expected. The exercise has been valuable in exposing some limits of the technology described earlier in this report. It was found that sulfonamidoglycosylation, either directly (cf. Scheme 22) or by the two-stage protocol employing a saccharide having an ethylsulfanyl substituent at the anomeric center (cf. Scheme 54), can be undermined if the combined steric demands of the donor and acceptor are too high. At the present time we have developed a sense as to when such coupling reactions may fail, but we do not have firm guidelines for predicting what levels of congestion may be tolerated for specific combinations of donors and acceptors. Clearly, such insight will be necessary if this already powerful method is to reach full fruition in applications to complex settings.

For the synthesis of the N3 antigen, we proceeded as described in Schemes 77 and 78.[142] Glycal 355 was used to construct the key building block 356, which was further converted to the complex azaglycosylation donor 357. The acceptor triol 358 bearing a levulinoyl protecting group was assembled from glucal and galactal. Fortunately, the 3',4',5'-triol in 358 was sufficiently noncongested that the coupling with 357 by direct "rollover" of the sulfonamido group was possible. Upon unveiling of the primary hydroxyl by hydrazine-induced cleavage of levulinoyl protecting group, acceptor 359 was in hand. Starting with D-glucal, following conversions that the reader will at this stage anticipate, donor 360 bearing a strategic *p*-methoxybenzyl protecting group at C4 of the GlcNAc ring was assembled (Scheme 78). The components met the anticipated requirements for coupling at the relatively unhindered primary acceptor site in 359 flanked by a free hydroxyl at C4'. Successful coupling was followed by acetylation of the axial C4' hydroxyl site on the galactose. We then exposed a unique acceptor site by cleavage of the PMB group (see asterisk 361). We had recog-



Scheme 77. Synthesis of the N3 antigen (part 1). CMPI = 2-chloro-1-methylpyridinium iodide, Lev = levulinoyl.



Scheme 78. Synthesis of the N3 antigen (part 2).

nized the steric congestion at this acceptor site and had accordingly simplified the demands on the coupling process through the use of a more reactive thioethyl donor. Indeed, **361** underwent successful coupling with donor **362**, fashioned from D-galactal, to produce the N3 glycal precursor **363** in 95% yield. We are currently completing the total synthesis of the N3 antigen and preparing useful N3 conjugates, which may find use as immunostimulants or as diagnostic agents.

This successful preparation of the protected N3 glycal **363** emphasizes an important dimension to the synthesis complex oligosaccharides not often recognized by students of general organic chemistry. Although the basic building blocks are in a broad sense generally defined in advance, there are many opportunities for ingenuity and creativity in how they are brought together. The problems can be quite challenging in the synthesis of compounds with increasing degrees of branching.

Note that in compound **361** one out of 19 potential hydroxyl groups[143] has been identified as the acceptor site. Moreover, it is located in a sterically congested region of the molecule. Yet coupling occurs smoothly because the acceptor center is being merged with a rather reactive donor (**362**). For those who are willing to labor at mastering the "grammar" of complex oligosaccharide synthesis, there is ample opportunity to author a new "literature" of large proportions.

11.3. Synthesis of Asialo-GM_1

In this vein we turn to our recently completed total synthesis of asialo-GM_1.[144] The biological rationale for conducting this project is clear: Al-Awqati and co-workers have implicated membrane-bound asialo-GM_1 as a primary infection site of cys-

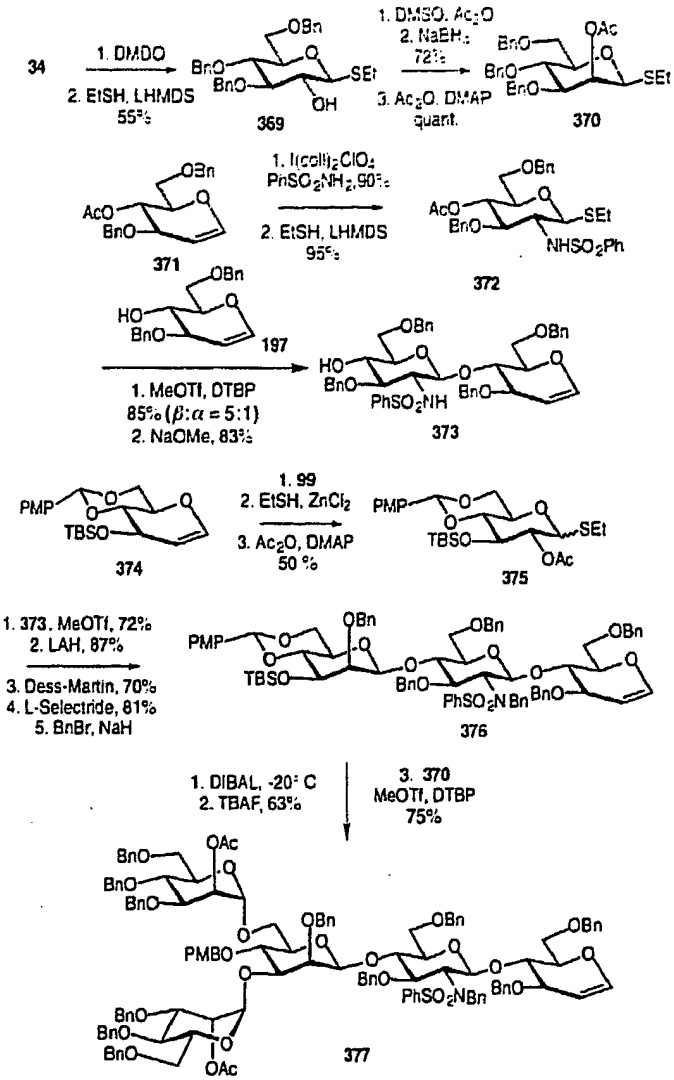
tic fibrosis associated pathogens.[145] Indeed, the interior GalNAc-Gal sector of asialo-GM_1 had earlier been claimed to be a general carbohydrate bioligand for pathogenic association in a variety of other infections.[146] Our hope is to generate soluble, cell-permeable versions of these substructures to study their potential as infection decoys.

The full power of azaglycosylation methodology has been brought to bear on the synthesis.[147] Our route started with the previously described **217**, which was suitably upgraded to produce the azaglycosyl donor **365** featuring a free axial hydroxyl in the donor ring (Scheme 79). As noted in the synthesis of the human breast tumor antigen (see Scheme 54), this arrangement tends to favor β-glycoside formation in the sulfonamidoglycosylation reaction. In the case at hand, **365** was coupled to the suitably differentiated and previously described glycosyl acceptor **213** to give **366**. This reaction constituted a severe challenge to our technology, as it required linkage at the very hindered axial bond of the lactal-derived construct. From this achievement, the steps to asialo-GM_1(**368**) followed the protocols that had been well worked out in previous demonstrations. Synthetic **368**, as well as other constructs derived from the terminal glycosidic sites does, indeed, bind to cystic fibrosis associated pathogens.[148]

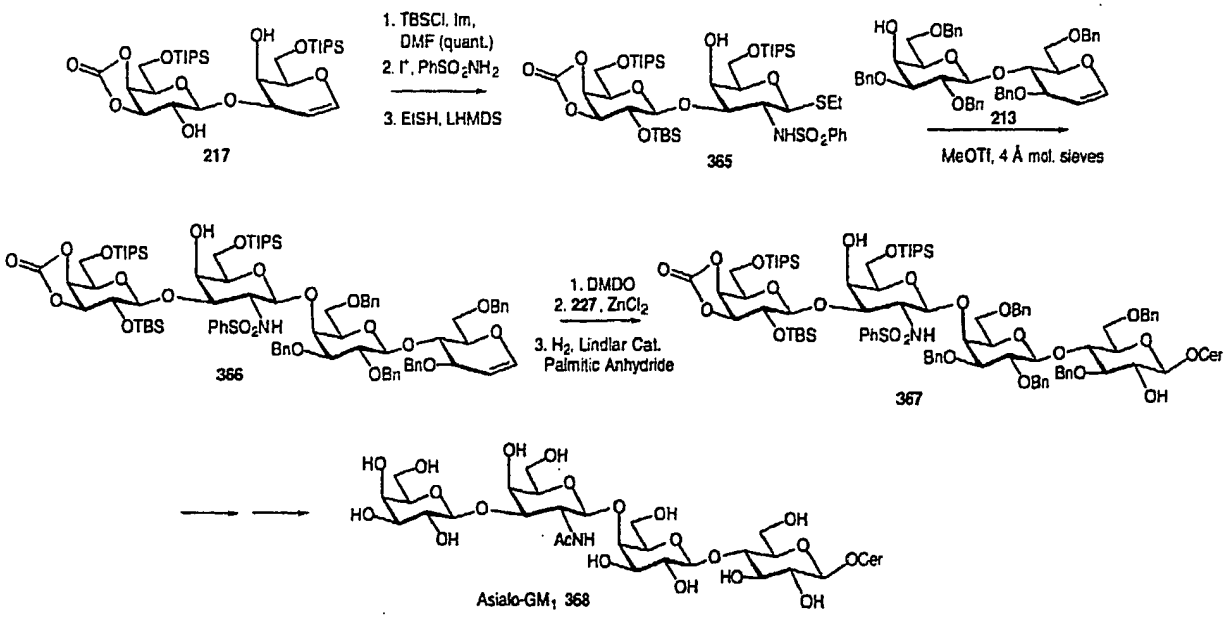
11.4. Synthesis of the Polysaccharide Core of a Glycopeptide

Our long-term program of synthesizing a natural-type (high mannose containing), fully competent and deprotected asparagine-linked glycopeptide has been progressing well (Scheme 80).[149] Thus, dibenzylglucal epoxide **34** was converted to the ethylsulfanyl-substituted **369** and further to the mannosyl donor **370** (cf. Scheme 30). The azaglycosyl donor **372** was fashioned from **371** as shown and successfully coupled to **197** to produce **373**.

A key element of our strategy was the use of the epoxide derived from glycal **374** protected at C4 and C6 by a *p*-methoxybenzylidene group. This compound was derived from D-glucal in the usual way. The epoxide derived from **374** was transformed to the ethylsulfanylglycoside **375**, which was coupled smoothly with **373** and **375**. Glycal **376** was eventually obtained. Reductive opening of the benzylidene linkage and desilylation pin-



Scheme 80. Synthesis of the polysaccharide core of a glycopeptide. LAH = lithium aluminum hydride. DIBAL = diisobutylaluminum hydride.



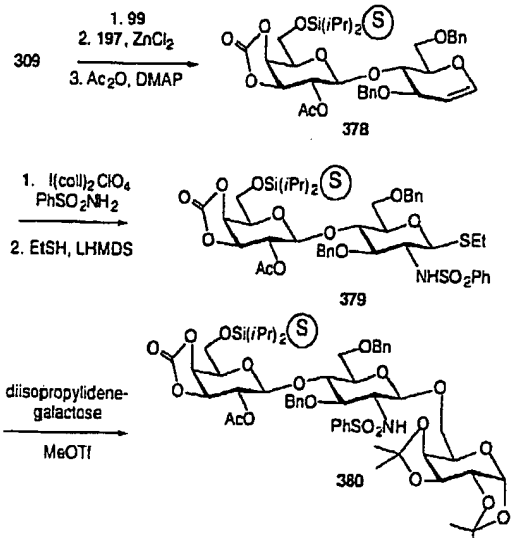
Scheme 79. Synthesis of asialo-GM_1. Im = imidazole.

pointed the α-mannosyl acceptor sites en route to the "high mannose region glycal" 377. The assembly of the full mannose region in the construct containing an explotiable glycal constitutes a significant advance. Upon refinement of this technology, the stage will be set for introduction of the asparagine-linked peptide by previously described methods (see Scheme 75).

11.5. Solid-Phase Synthesis of a 2-Sulfonamido-2-Deoxyglycoside

Of course, our long-term goal is to synthesize the entire glycopeptide, including the mannose-rich region, on a solid support. The carbohydrate section of the glycopeptide would, eventually, culminate in presentation of blood group or tumor antigen epitopes at the nonreducing end of the construct. Previously, we had noted that azaglycosylation was not successful in our solid-phase protocols, save for introduction of an azido group at the anomeric center (see discussions associated with Schemes 72 and 73).

A significant advance in our technology has now arisen from the demonstration that the polymer-bound 378, prepared from the previously described 309 in the usual way, can be used to furnish a competent azaglycosyl donor (Scheme 81).[150] Thus,



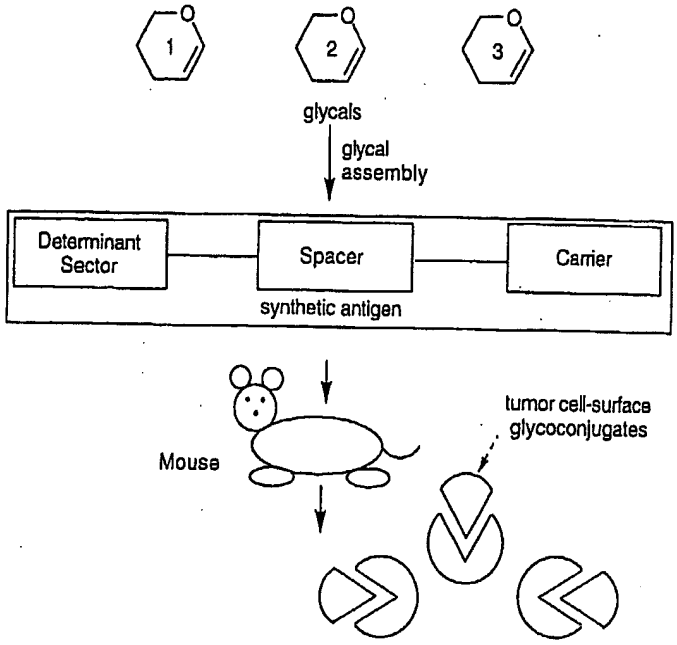
Scheme 81. Lactosamine synthesis on a solid support.

378 was converted to the polymer-bound donor 379, which reacted with solution-phase diisopropylidenegalactose to produce 380. Thus, though the generality of azaglycosidation of polymer-bound donors is not yet fully established, certainly the prospects for achieving a strictly solid-phase synthesis of glycopeptide-linked tumor antigens are now quite promising.

11.6. Preclinical Assessments

At present we are focusing on protein conjugates of the human breast tumor (MBr1) and Le^y antigens. In each case, synthetic conjugates bind to appropriate antibodies. These studies clearly implicate the carbohydrate sectors as the key immunological recognition sites. Mapping efforts are well underway to ascertain the key contributions to binding in the carbohydrate domain.

Most encouraging is the fact that our fully synthetic constructs in both series, when injected in mice, generate antibodies that bind in vitro to target cell lines (Scheme 82). These promising findings invite the next question, whether such constructs will provide clinically useful immunological protection or even regression in a human setting. The protocols needed to put such large and important questions to the test are now being developed.



Scheme 82. Inoculation of mice with synthetic antigens. Mouse antibodies bind to tumor-transformed cell lines.

12. Conclusions and Futuristic Perceptions

In this article we have shown, by example, the power of glycal assembly. Of course, ours is only one of several laboratories addressing the synthesis of complex oligosaccharides. We cannot claim these methods to be indispensable; all of the target molecules described in this review could probably have been reached by other coupling methods or assembly strategies. A fair number of the syntheses shown here have in fact been achieved through more conventional carbohydrate chemistry. We do, however, feel that glycal assembly may offer large advantages in synthetic conciseness. These stategies may obviate many of the onerous protecting group manipulations that have dominated this field. Glycal assembly has stimulated the development of new coupling technologies, and the methods are constantly improving. Those who maintain an open mind about carbohydrate technology would be well advised to consider ways in which glycals might be useful for their purposes.

We are confident that solid-phase oligosaccharide synthesis, our methods and others,[138] will be expanded, and that substantial progress in this regard is in the offing. We expect major new advances in glycopeptide synthesis to be realized by our methods, by other chemical methods, and, indeed, by enzymatically assisted methods. Certainly, the goal of adapting solid-phase methodology to include assembly of the mannose-rich regions of glycopeptides is high on our list of priorities.

Ultimately, the test of the success at the chemical level will be whether complex oligosaccharides and glycoconjugates can be synthesized effectively by even nonspecialists. Despite the success of the methods described here, this is currently far from the case and further developments are needed. The challenges and the opportunities of oligosaccharide synthesis are immense and many solutions are possible, including some which await creative formulation by future practitioners.

We venture the opinion that even as progress is made on the chemical issues, the "really decisive" terrain may be shifting. In the past chemists have lavished a great deal of time and energy in developing a host of glycosylation methods and strategies. These advances have spawned a new and very serious challenge—the conceptualization of valuable targets. Though many unsolved chemical problems continue to arise, the time is already at hand to prepare and evaluate new constructs to test important possibilities in structural biology, immunology, and medicine. In our own work it is only in the last year that our glycal methodology has produced ample amounts of carrier protein conjugated carbohydrate-based tumor antigens for preclinical and, hopefully, clinical evaluation. At this time no one can safely predict the full impact of such carbohydrate–protein constructs on either the diagnosis or the treatment of cancer. The most we can safely assert is that this clinical issue will be probed in detail. Thus, the largest challenges may now lie in choosing synthetic targets and in marshaling the multidisciplinary resources for systematic evaluation of the products of such efforts.

We close this review with some thoughts concerning life on the chemistry–biology frontier. In this account we have shown by historical progression how our laboratory, starting with fascinating problems in the field of "small molecule" natural products, has become involved in issues of tumor expression and tumor immunology. One of the singular contributions which we and like-oriented research groups bring to such coalitions is a sensitivity for precisely defined structures. When collaborating with biologists in identifying bioactive compounds and charting their functions, the chemist insists that the compounds in question be demonstrably pure and that the structural assignments, down to each stereogenic center, be corroborated. But chemistry's contribution to the enterprise is certainly more than restraints arising from insistence on thoroughness and intellectual exactitude. Methodical building upon the principles of our science leads to the magic of synthesis—with its unique capability to prepare molecules of virtually any shape and juxtaposition of functional groups. Creative synthesis is the indispensable talent that the chemist will bring to the many exciting struggles and opportunities in the future.

The research reviewed in this paper was possible only through the dedication, enthusiasm, and creativity of scores of co-workers, whose names are acknowledged on the publications cited from our laboratory. In a few instances we identified individual colleagues who were involved at particularly sensitive points of the journey. However, it is to all our associates on these papers, and to other members of the laboratory (who were available for the extensive discussions that helped to fashion these experiments) to whom the program is lastingly indebted.

Needless to say, external funding was also necessary! This support was provided largely by the National Institutes of Health (AI 16943, CA 28824, HL 25848). In addition, M. T. B. gratefully acknowledges the NIH for a Postdoctoral Research Fellowship (GM 16291). We also note and express our thankfulness for generous inputs over these twenty years from various public-spirited supporters in the pharmaceutical industry, particularly the Merck Corporation and, more recently the Pfizer Foundation for Graduate Fellowships.

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